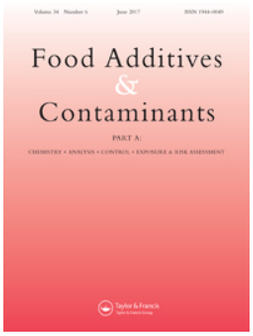

Taylor & Francis
Taylor & Francis Group

Food Additives & Contaminants: Part A

ISSN: 1944-0049 (Print) 1944-0057 (Online) Journal homepage: https://www.tandfonline.com/loi/tfac20

Exposure assessment of epoxy fatty acids through consumption of specific foods available in Belgium

Edward Mubiru, Liesbeth Jacxsens, Antonios Papastergiadis, Carl Lachat, Kshitij Shrestha, N. H. M. Rubel Mozumder & Bruno De Meulenaer

To cite this article: Edward Mubiru, Liesbeth Jacxsens, Antonios Papastergiadis, Carl Lachat, Kshitij Shrestha, N. H. M. Rubel Mozumder & Bruno De Meulenaer (2017) Exposure assessment of epoxy fatty acids through consumption of specific foods available in Belgium, Food Additives & Contaminants: Part A, 34:6, 1000-1011, DOI: 10.1080/19440049.2017.1310399

To link to this article: https://doi.org/10.1080/19440049.2017.1310399

FOOD ADDITIVES & CONTAMINANTS: PART A, 2017
VOL. 34, NO. 6, 1000–1011
https://doi.org/10.1080/19440049.2017.1310399

Taylor & Francis
Taylor & Francis Group

Exposure assessment of epoxy fatty acids through consumption of specific foods available in Belgium

Edward Mubiru, Liesbeth Jacxsens, Antonios Papastergiadis, Carl Lachat, Kshitij Shrestha, N. H. M. Rubel Mozumder and Bruno De Meulenaer

nutriFOODchem Unit, Department of Food Safety and Food Quality (Partner in Food2Know), Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

ABSTRACT

Epoxy fatty acids (EFAs) are secondary oxidation products formed from unsaturated fatty acid hydroperoxides. Seventeen food categories were analysed for C18 monoEFAs of food products available on the Belgian market. A quantitative exposure assessment was performed based on deterministic and probabilistic approaches combining these concentration data with consumption data obtained from the Belgian National Food Consumption Survey of 2004. A preliminary evaluation of any potential risk related to the intake of the studied EFAs through the studied foods was performed by applying the threshold of toxicological concern (TTC) concept. Three food categories out of 17 foods, mayonnaise, butter–margarine and ready-to-eat meals were found to contribute most to the intake of EFAs. According to probabilistic determination, these foods had P50 intakes of 0.4085, 0.3328 and 0.2997 mg kg^{-1} bw day^{-1} respectively. They had P99.5 intakes of 3.7183, 2.7921 and 38.6068 mg kg^{-1} bw day^{-1} respectively. The intake below the TTC was from the consumption of cooked meat, smoked salmon and raw cured ham, with P50 intakes of 0.0006, 0.0007 and 0.0011 mg kg^{-1} bw day^{-1} respectively, and the other foods were above the TTC. Based on the TTC concept, a risk to human health could be identified related to the consumption of cheese, snacks foods, plant oils, French fries, dry nuts, chips, cured minced raw meat, cookies, fresh and frozen salmon and bacon.

ARTICLE HISTORY
Received 21 December 2016
Accepted 8 March 2017

KEYWORDS
Lipid oxidation; fatty acid methyl esters; threshold of toxicological concern; risk assessment; exposure assessment

Introduction

Lipid oxidation is a deterioration process that results in food spoilage and potential production of toxic compounds. Some of the compounds formed have unfavourable flavours that make food unacceptable and unfit for human consumption. Lipid oxidation is common to monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs); the amounts of MUFAs and PUFAs present will determine how prone the food is to lipid oxidation. Lipid oxidation can proceed by auto-, thermo-, photo- and enzymatic mechanisms (Frankel 2005). The amounts of oxidation products formed will depend on the mechanism at play and intrinsic and extrinsic factors present in the food such as the amount of PUFAs, antioxidant or pro-oxidants, storage conditions etc. The reactions proceed via formation of primary products (hydroperoxides), which later break down to various secondary products (Steele 2004). Epoxy fatty acids (EFAs) are among the secondary lipid oxidation products formed. EFAs are a group of straight-chain aliphatic monocarboxylic acids with an oxirane ring in their structure (Swern 1955). They form due to incorporation of an oxygen moiety into the normal fatty acid chain by direct addition to the double bond or by cyclisation of an alkoxy radical (Frankel 2005; Schaich 2005).

Long-chain EFAs occur naturally in oil seeds and in cutins or may be produced from hydroperoxides during storage and oil refining. They are also manufactured in large amounts by epoxidation of appropriate alkene esters for industrial purposes (Gunstone & Jacobsberg 1972; Gunstone & Schuler 1975). EFAs are potential toxic compounds to humans, which is attributed to their high reactivity because of the presence of the oxirane ring in their structure. This, under appropriate conditions, can react with proteins and DNA, consequently leading to structural damage and alteration of their functionality (Greene, Williamson, et al. 2000; Harwood

CONTACT Bruno De Meulenaer Bruno.DeMeulenaer@UGent.be Ghent University, Coupure Links 653 B-9000, Belgium

et al. 2007). Specifically, *cis*-9,10-epoxystearate and its isomer *cis*-12,13-epoxy oleate are leukotoxic and isoleukotoxic respectively. The EFAs have been reported to have low absorptivity in the body, a property which suggests that high levels may be detected in the colon. Although there has not been a strong link of EFAs to health risks, some studies have suggested that they may cause cancers (Wilson et al. 2002).

Although some studies have reported EFAs to be stable and only open in acid-catalysed reaction media (Wilson et al. 2002), which is more likely to happen in the stomach, the epoxy hydrolases are able to add water to EFAs to yield vicinal diols, which account for their observed toxicity (Greene, Newman, et al. 2000). The hydroxyl diols are more toxic than the EFAs themselves. However, in a study with labelled EFAs, they were absorbed intact and it was observed that mono-EFAs were more absorbed than diepoxy fatty acids (Wilson et al. 2002). This is interesting since most of the studies quantified only mono-EFAs and it has been reported that high levels of these fatty acids occur in foods (Fankhauser-Noti et al. 2006; Mubiru et al. 2013). During toxicity studies, EFAs of C18–C20 were observed to exhibit soluble epoxide hydrolase (sEH)-dependent toxicity (Greene, Newman, et al. 2000). Based on structural and functional similarity, 12 EFA isomers derived from oleic, linoleic and linolenic fatty acids are assumed to possess the same toxic effects. It has also been reported that the sulphydryl (SH) and amino groups in some proteins can react with the epoxy group thus later lead to loss of protein functionality and availability (Wilson et al. 2002).

Recently there have not been many toxicity studies performed *in vivo* except for one study done on mice (Zheng et al. 2001). However, a cytotoxicity study by Greene, Newman, et al. (2000) performed *in vitro* on *Spodoptera frugiperda* (Sf-21) cells, with human soluble epoxy hyrolase (hsEH) and β-galactosidase (Lac Z) as control enzymes, showed that EFAs were toxic to hsEH with LC_{50}'s of 0.112 and 0.428 mM for (*Z*)-9,10-epoxyoctadecanoic acid methyl ester and (*Z*)-6,7-epoxyoctadecanoic acid methyl ester respectively. Surprisingly, the toxicity to the cell increased from C11 to C18 and later decreased with an increase in the chain length. It was confirmed that neither EFAs were toxic for β-galactosidase (Lac Z), suggesting that diol formation is a detoxification step in some cases (Greene, Newman, et al. 2000).

To date there are no available robust toxicological data of EFAs and there is limited knowledge concerning the dietary exposure of humans to these compounds except for an experimental study performed on women (Wilson et al. 2002). In the absence of this specific toxicological data, a risk assessment can be done based on the threshold of toxicological concern (TTC) concept. The concept establishes human exposure thresholds below which there would not be an appreciable risk to human health. The TTC concept is a useful tool for risk characterisation of chemicals of known structure to which humans are exposed at low levels (Kroes et al. 2004; Rennen et al. 2011; Hennes 2012). Application of the TTC principle as a preliminary risk-characterisation approach for comparing potential exposure and the TTC threshold, based on the substance's structure, could help avoid the need for further detailed assessment of chemicals to which humans are exposed at low levels. It is intended to avoid unnecessary extensive toxicity testing and safety evaluation which are always complex, thus saving resources (Kroes et al. 2005). It is only when the values obtained after the application of the TTC concept are high that a full-risk characterisation can be recommended.

Exposure assessment studies are missing on secondary lipid oxidation products which are potentially toxic to humans except a recent study done on malondialdehyde (MDA), 4-hydroxy-2-hexenal (HHE) and 4-hydroxy-2-nonenal (HNE) in our laboratory (Papastergiadis et al. 2014). During this study, it was reported that, based on the TTC concept, consumption of the specific foods did not pose a risk to most Belgian consumers. However, exposure to MDA through consumption of especially dry nuts was significant compared with other aldehydes, and 4% of the population consuming cured and minced raw meat products were found to be at a risk of HNE exposure (Papastergiadis et al. 2014). Risk-assessment studies are based on reliable intake data which are generated from accurate analytical determination of analytes. The objectives of this study, therefore, were to determine EFAs in specific foods available on the Belgian market and then to perform a quantitative exposure assessment of

dietary intake of the EFAs for the Belgian population (both consumers and the total population) using the food-consumption data of the Belgian National Food Consumption Survey (BNFCS) 2004. Finally, evaluation of the potential risk due to total EFAs was performed by comparing the estimated intakes with the corresponding TTC of the EFAs (class III) and discussed against the earlier reported findings of other secondary lipid oxidation products (MDA, HHE and HNE) by Papastergiadis et al.

Materials and methods

Supplies and reagents

Methyl *tert*-butyl ether (MTBE) and 3-chloroperoxybenzoic acid (70–75%) were purchased from Acros Organics (Geel, Belgium). Sodium methoxide solution (25% w/v), silica gel 60 for column chromatography (particle size = 0.063–0.100 mm) and were bought from Sigma Aldrich (St. Louis, MO, USA). Methyl *cis*-10-heptadecenoate (C17:1), and a mixed FAME GLC 68D standard were obtained from Nu-Chek-Prep. Inc. USA (Elysian, MN, USA); all other chemicals and reagents were of analytical grade and obtained from the reliable sources.

Sampling plan for EFA determination in foods

The choice of study foods (Table 1) was adapted from a recent study done by Papastergiadis et al. (2014). This was a directed or risk-based sampling where specific foods were sampled according to their potential risk to be contaminated with the secondary oxidation products. Fat content and the degree of unsaturation of the fatty acids were taken into account, thus foods were divided into three groups: foods of plant origin, foods of animal origin and chilled cooked meals containing multiple ingredients (Papastergiadis et al. 2014). Two other food groups, mayonnaise, and butter and margarine, were included based on their importance in the normal diet of most Belgian consumers (Temme et al. 2010). A total of 390 samples were purchased from supermarkets in the region of Ghent, Belgium, excluding French fries and fried snacks which were purchased from local fast-food restaurants. Foods were purchased and always analysed immediately upon delivery to the laboratory. For purposes of obtaining a representative sample, all the components of the foods were homogenised using a Waring stick blender prior to taking the required weights before analysis.

Determination of EFAs in foods

Base-catalysed transmethylation procedures and the SPE separation steps have been previously described (Mubiru et al. 2013, 2014). Briefly, 5 ml of MTBE were added to the extracted oil in a 25-ml glass tube. Then, 2.5 ml of a 0.2 M sodium methoxide solution in methanol were added and vortexed for 1 min, and allowed to stand at RT for 2 min. Then, 0.17 ml of 0.5 M sulphuric acid were added and the mixture vortexed for a few seconds. Finally, 5 ml of water were added and vortexed for 2 min. The resultant FAME was dissolved in 2 ml of *n*-hexane–diethyl ether (98:2, v/v) and loaded onto a silica column for

Table 1. Description of the foods analysed.

Food group	Food category	Food description
Foods of plant origin	Chips	Chips with different flavours salted and non salted
	Cookies	Biscuits, waffles, cakes, speculoos
	Dry nuts	Roasted, peeled and non salted peanuts, walnuts, hazelnuts, almond, pistachios, cashew
	French fries	Fries with different sauces (mayonnaise, tartar, andalouse)
	Plant oils	Refine (corn, sunflower, arachis, colza, soya, salad, frying, a mixture) and extra virgin oils
Foods of animal origin	Bacon	Salted and smoked and non-smoked
	Butter	Salted and non salted
	Cheese	Gouda and cheddar
	Cooked meat	Grilled or boiled Paris sausage, frankfurter
	Mayonnaise	Normal, light and similar sauces
	Milk	Full fat pasteurised and sterilised milk
	Raw ham	Salted and ripened
	Cured minced raw meat	Dry sausage, salami
	Smoked salmon	Filleted and stored at 4°C
	Fresh and frozen salmon	Stored at −20°C
	Snack foods	Boulet, chicken finger, hamburger, fish sticks
Cooked chilled food	Ready-to-eat meals	Lasagna, spaghetti, chicken fillets, fried rice, stewed beef, pasta, pork meat balls, burgers

pre-separation. After SPE the epoxy fraction was dissolved in 300 µl of iso-octane ready for GC-FID analysis. The GC analysis for the fatty acids was performed on a GC-FID Agilent 6890N series gas chromatograph (Agilent, Foster City, CA, USA). The samples were dissolved in isooctane, and 0.1 µl was injected directly into the capillary column using a cold on-column injector; separation was performed in a CP-Sil 88™ for FAME (60 m × 0.25 mm ID) capillary column coated with a 0.2-µm film. An Agilent deactivated fused silica pre-column 3 m × 0.25 mm i.d. (Machelen, Belgium) was fitted to protect the column. The oven temperature programme was set as follows: 50°C hold for 4 min, then ramp to 225°C at 22°C min^{-1}, and hold for 25 min. The flame ionisation detector temperature was set at 350°C. The detector flow rates for hydrogen, air and helium (make-up) were 40, 400 and 20 ml min^{-1} respectively. Helium flow rate as a carrier gas was 1 ml min^{-1}. Confirmation of individual epoxy FAMEs was carried out by comparison of retention times with those of authentic standards of an EFA mixture and the fatty acid methyl ester mixture (GLC 68D, Nu-Chek Prep).

The LODs of the method considered were determined previously (Mubiru et al. 2013, 2014). The LOD of EFAs in oils was found to be 2.8 µg g^{-1} of oil. The LOD of EFAs for dry nuts, potato crisps, French fries and cookies was taken to be 10.2 µg g^{-1} of sample. The LOD for mayonnaise, margarine–butter, cheese and milk was 1.7 µg g^{-1} of sample. The LODs concerning all the meat products and ready-to-eat meals were taken to be 5.2 µg g^{-1} of sample.

Consumption data

Food-consumption data were obtained from the Belgian National Food Consumption Survey (BNFCS) of 2004. The aims and methodology are described by De Vriese et al. (2005). Dietary intake was collected from 3245 individuals aged 15 years and above residing in Belgium (Vandevijvere et al. 2009). The survey was based on two non-consecutive 24-h recalls combined with a self-administered food-frequency questionnaire. Consumption information of the specific foods was extracted from the BNFCS database based on their description (food category and food name data) (Table 1). The usual food intake was determined from the total dataset by correcting for intra-person variability using the multiple source method (MSM) program (Harttig et al. 2011; Haubrock et al. 2011; Institute of Human Nutrition Potsdam-Rehbrücke (DIfE) 2013). All subjects were considered habitual consumers of the foods, and food intake data were expressed in kg of food bw day^{-1} using the body weight (bw) data collected in the survey. The food-consumption database used does not contain information about cooked chilled meals (bought as ready-to-eat products), as such a discrete function was used to model their intake which was obtained from a consumer survey data conducted by Daelman et al. (2013) where a total of 679 individuals aged 15 years and above were interviewed on their consumption behaviour of ready-to-eat meals during the spring of 2011 in Belgium. This survey was based on frequency of consumption where respondents had to answer the question 'How often do you eat a ready-to-eat meal?' Seven answers were possible: 5–7 times a week, 2–4 times a week, once a week, 3–5 times a month, once a month, once a year and never. These responses were first converted to a daily consumption by a conversion factor (i.e., 5–7 times a week corresponded to 1/day; once a week corresponded to 1/7 days; 3–5 times a month corresponded to 4/30 days, once a month corresponded to 1/30 and once per year corresponded to 1/365), followed by a multiplication of the average weight of all analysed products. The outcome was divided by the average body weight of 60 kg (Kroes et al. 2005). At this point it should be mentioned that the total weight of each item was considered as a whole single-personal portion (Daelman et al. 2013; Papastergiadis et al. 2014).

For those data shown in Table 4, a risk discrete function has been attributed, with the first argument in the discrete function expressing the consumption of ready-to-eat meals being the set of possible values (relative respondents); the second is the set of corresponding probabilities. By application of this function, the consumption dataset of kg (ready-to-eat meals) kg^{-1} bw day^{-1} was obtained. To obtain information about the total intake of the population including consumers and non-consumers, the IF (a logical function which checks whether a condition is met and returns one random number between 0 and 1) function was applied to the consumers' distribution; since the

percentage of consumers was known, random intakes could be returned that were used to infer the population intake (Medeiros Vinci et al. 2012).

Exposure assessment

During EFA analysis, no concentrations were below the LODs (non-detects), so no data censoring was necessary during exposure assessment (Kroes et al. 2002; Picot & Roudot 2012). For the exposure assessment, foods were divided into 17 categories: plant oils, dry nuts, potato crisps, French fries, cookies, fried snacks, frozen and fresh salmon, smoked salmon, full-fat milk, cheese, cured and cooked meat products, bacon, cured raw ham, cured minced raw meat products, mayonnaise, margarine and butter, and ready-to-eat meals (Table 1).

Deterministic exposure assessment

Dietary exposure of the consumers and total Belgian population to the EFAs was initially performed using the deterministic approach. This refers to using a model with no uncertainty and variability consideration, which may not give a full estimate of the intakes. Estimated intakes were calculated by multiplication of a fixed mean of EFA contamination data with the mean, maximum or P99.5 percentile of the consumption data of each food category. This approach was deemed necessary to minimise the risk to consumers (Vromman et al. 2010). Deterministic analysis could not be applied to the cooked chilled meals because of the nature of the consumption data.

Probabilistic exposure assessment

Distribution fitting and Monte Carlo simulations were performed with the @Risk for Microsoft Excel software version 5.7.1 (Palisade Corporation, Ithaca, NY, USA), with 50,000 iterations and three simulations. Best-fit distributions for both consumption and contamination data of each food category were determined (Table 4). The best-fit distributions were defined based on chi-square statistics, probability–probability plots (P-P) and quantile–quantile plots (Q-Q). Distribution fitting was feasible when at least five positive data were available (Vose 2008; Medeiros Vinci et al. 2012). Monte Carlo simulations were performed for each food category to develop the exposure model considering uncertainty and variability. The probability of existence of EFAs in the different foods, their levels in those foods and the probability of human exposure were all outputs of the mathematical model. The estimated daily intake (mean, standard deviation, maximum and percentiles) was expressed in mg kg^{-1} bw day^{-1} of EFA.

Threshold of toxicological concern

Chemicals were classified into three classes according to a Cramer decision tree and the TTC values for chemicals belonging to Cramer classes I, II and III were 1800, 540 and 90 μg person day^{-1} respectively taking a normal body weight to be 60 kg (Cramer et al. 1978). The decision tree comprises a sequence of questions such that compounds with structures indicative of a high potential for toxicity are assigned to structural class III (Kroes et al. 2004). Classification of the compounds based on the Cramer decision tree was carried out with Toxtree (v. 2.5.4) software available online from the German Institute of Human Nutrition Potsdam-Rehbrücke. EFAs were confirmed to be grouped under class III.

Statistical analysis

One-way analysis of variance (ANOVA) was applied to detect differences in the mean of the total concentrations of the EFAs between different food categories. In case of lack of homogeneity of data, a logarithmic transformation was applied prior to statistical analysis to achieve homoskedasticity. For significant differences, a Student–Newman–Keuls test of mean comparisons was applied and a p-value of 0.05 was used. Calculations were performed with SPSS 22 statistical package (IBM, SPSS, Inc.).

Results and discussion

Occurrence of EFAs in foods on the Belgian market

Results of the concentrations of C18 EFAs for a total of 390 food samples analysed are presented in Table 2. The total EFA content is shown, but in most cases the total comprises 12 mono-EFA isomers analysed from the different food matrices. The method used to analyse EFAs in food matrices and oils was previously validated in-house (Mubiru et al. 2013, 2014), and it should be noted that free EFAs cannot be analysed by these methods. Only EFAs that are still attached to a

Table 2. Total C18 epoxy fatty acid concentrations (mg kg^{-1}) in the different foods analysed.

Food categories	*N*	Mean	SD	Maximum
Chips	24	297.71^{def}	218.46	872.27
Cookies	29	148.22^{cd}	43.65	263.16
Dry nuts	24	686.98^{g}	513.19	2.117.43
French fries	19	128.50^{c}	92.42	360.32
Plant oils	36	410.68^{def}	427.67	1.933.26
Bacon	25	73.49^{b}	53.52	187.74
Butter	27	408.93^{f}	229.30	143.34
Cheese	24	309.59^{ef}	138.96	668.54
Cooked meat	22	12.54^{a}	8.17	34.72
Mayonnaise	25	309.61^{def}	318.47	1.754.00
Milk	17	40.22^{b}	24.37	118.75
Raw ham	22	65.85^{b}	68.41	225.95
Cured minced raw meat	26	208.98^{cde}	106.48	488.59
Smoked salmon	21	69.12^{b}	40.33	146.24
Fresh and frozen salmon	9	82.25^{b}	62.23	178.73
Snack foods	20	186.06^{cde}	65.96	325.27
Ready-to-eat meals	20	59.37^{b}	38.56	150.85
Total	390			

Note: Values with different superscripts in a column are significantly different $p < 0.05$.

triglyceride backbone are analysed. EFAs were detected in high amounts in most of the food samples, especially those in the plant foods category; there were no non-detects (below the LODs) in all the samples. According to published data (Fankhauser-Noti et al. 2006; Mubiru et al. 2013, 2014), EFAs can be found in food samples up to mg levels and 12 C18 isomers are known to occur of which the *cis*-9,10-epoxystearate is the most abundant. All the foods could be categorised into three groups according to the amount of EFAs determined: low EFA (< 100), medium EFA (100–200) and high EFA (> 200) mg kg^{-1} of sample.

Generally, animal-based foods had low levels of EFAs compared with plant foods ranging from 12 to 82 mg kg^{-1} of sample and specifically these were found in cooked meat, chilled cooked meals, raw ham, smoked salmon, fresh and frozen salmon, milk and bacon. These low levels seem to be related to the types of fatty acids that are common in animal-based foods as they have a low content of C18:1 and C18:2 fatty acids (Woodgate & van der Veen 2014). Foods that had more than 200 mg kg^{-1} of EFAs were plant oils, butter and margarine, cheese, mayonnaise, chips, cured minced raw meat (salami), and cookies. Cured minced raw meat, a category of sausages and salami, had a higher incidence of EFAs up to 209 mg kg^{-1} of sample compared with the raw meat which had 66.9 mg kg^{-1}; this may be linked to the further ripening and drying treatment which is given to the meat during processing.

Among the plant-based category foods, dry nuts had the highest EFA content of 687 mg kg^{-1}. This high incidence may not be surprising since some oil seeds have been reported to have naturally occurring EFAs to a level of 60 to 80% vernonia oil (Spitzer 1999; Gunstone et al. 2012). This observation could partly be attributed to the high content of oleic and linoleic fatty acids found in most plant-based foods considering the fat and fatty acid content. Coupled to this observation, some of the seeds could further be processed by roasting and salting which may affect the EFA content. However, any correlation between roasting and its effect on the production of EFAs is not known, but it may be that further treatments of nuts could increase the incidence of EFAs since high temperatures are involved which may accelerate the rate of lipid oxidation.

Although butter is obtained from milk which had a low EFA content, the butter–margarine category had a content of EFA as high as 409 mg kg^{-1} on average. This could mainly be because margarine, an alternative to butter, is made by hydrogenation of plant oils which may already have a high epoxy content. However, it was not also possible to explain the relationship between EFA content in cheese and milk vis-à-vis the fat or fatty acid content, but irrespective of the fatty acid content, foods that are further processed had higher EFAs when compared with those that remain in the unprocessed form.

Most of the vegetable oils used in this study were fresh and refined with very low initial peroxide values (data not shown), possibly because of the refining process. It was observed that high standard deviations occurred in some datasets. This may be an indicator of the heterogeneity of the distribution of EFAs in the different food matrices. This problem can be overcome by increasing the number of samples that can help to reduce this cause of variation and increase the validity of the results (Kroes et al. 2002).

Consumption data

The total sample of 3245 individuals included in the BNFCS provided useful information on the consumption of the analysed samples by the Belgian population. The highest number of consumers was obtained from the butter–margarine food group, with 91% consumers. This high percentage may be attributed to the wide range of foods that fall under this category, especially the margarine, which all contribute to the daily

intake. The rest of the foods were grouped per the percentage of consumers shown in Table 4. Foods consumed by more than 20% of the Belgian respondents were milk (64%), mayonnaise (44%), cheese (45%) and plant oils (36%). Foods consumed by between 10% and 20% of consumers included French fries (25%), cured processed meat products (14%), cookies (12%), cured unprocessed meat products (11%), cooked and processed meat products (10%) and chips (9.6%). Foods found to be consumed by fewer than 10% of the individuals included bacon (9%), dry nuts (5%), frozen and fresh salmon (4%), smoked salmon (4%) and fried snacks (3%). Ready-to-eat meals were consumed by 78% of participants (Daelman et al. 2013).

Deterministic exposure assessment

Table 3 presents the estimated intakes (mg kg^{-1} bw day^{-1}) by Belgian adult consumers for the different foods. Initial estimation of the exposure to EFAs through the consumption of each individual food category was based on a deterministic approach. Deterministic analysis for the chilled cooked meals could not be applied due to the nature of the available consumption data. Food categories that had the lowest contribution to the mean intake of EFAs were cooked meat products followed by smoked salmon, raw cured ham, bacon, cookies and fresh and frozen salmon with levels of 0.0011, 0.0013, 0.0024, 0.0046, 0.0058 and 0.0068 mg kg^{-1} bw day^{-1} respectively. Since no toxicological reference values exist for EFAs, the TTC of 0.0015 mg kg^{-1} bw day^{-1} was used to estimate the risk to human health. This is the USFDA's 'threshold of regulation' set value, which is defined as 0.5 ppb in the diet corresponding to 1.5 µg/person/day (USFDA 1995). The lowest exposure to EFA was through consumption of cooked meat. This can be attributed to the lowest mean contamination levels (12.54 mg kg^{-1}) combined with the low average daily meat intake (1.0×10^{-4} kg of food kg^{-1} bw day^{-1}). Exposure due to the consumption of milk appears higher even when the EFA content is low (mean concentration of 40 mg kg^{-1}); this is mainly because higher amounts are consumed daily by individuals (mean consumption 8×10^{-3} kg of food kg^{-1} bw day^{-1}).

Table 3. Deterministic approach-estimated intake of epoxy fatty acid for different foods (mg kg^{-1} bw day^{-1}).

Food categories	Mean	P99.5	Maximum
Chips	0.0128	0.3434	2.6425
Cookies	0.0058	0.1478	0.8570
Dry nuts	0.0204	0.6198	9.9099
French fries	0.0413	0.8774	1.8511
Plant oils	0.1196	1.6808	2.6300
Bacon	0.0046	0.1716	0.4262
Butter	0.4652	1.6243	2.9110
Cheese	0.1400	2.1054	5.5076
Cooked meat	0.0011	0.1716	0.4262
Mayonnaise	0.5887	1.7289	2.0754
Milk	0.3156	3.5879	8.8297
Raw ham	0.0024	0.0584	0.4254
Cured minced raw meat	0.0112	0.2711	0.8130
Smoked salmon	0.0013	0.0579	0.3388
Fresh and frozen salmon	0.0068	0.4164	1.1062
Snack foods	0.0089	0.2744	3.0166
Ready-to-eat meals	n.a.	n.a.	n.a.

Note: n.a., Not applicable.

The highest exposure for consumers was found to originate from the consumption of mayonnaise, butter, fried snacks, cheese and plant oils. The highest exposure to the EFAs is specifically attributed to mayonnaise and butter as the most significant sources of mean EFA intake estimated at levels of 0.589 and 0.465 mg kg^{-1} bw day^{-1} respectively. Although dry nuts are not consumed in large amounts (3×10^{-5} mg kg^{-1} bw day^{-1}), a high mean daily intake of 2×10^{-2} mg food kg^{-1} bw day^{-1} was observed and this is due to the high contamination levels (687 mg kg^{-1} of nuts). Concerning chips and cookies, which are important snacks among the consumed foods in Belgium even with preschool children (Huybrechts et al. 2008), the exposure was approximately nine and four times higher than the TTC. Considering P99.5 and maximum intake of EFAs in all the foods using a deterministic approach, high levels of exposure were registered and it confirmed that the portion of consumers who are consuming highly contaminated specific foods in large amounts are more exposed to high amounts of EFAs than average consumers. This maximum intake of EFAs in all foods is of concern, and because there was exceedance of the intake by the consuming population, the use of a probabilistic approach was deemed necessary to calculate further the exposure and the risk to consumers.

Probabilistic exposure assessment

More accurate determinations of exposure were obtained during probabilistic analysis since each possible value a variable can take and the possible probabilities of its occurrence are taken into account (Vose 2008). Consumption and contamination data for the different food categories were

Table 4. Best-fit distribution functions used, minimum, mean and maximum concentrations of epoxy fatty acids (mg kg^{-1}) and food intakes of Belgian consumers (mg kg^{-1} bw day^{-1}).

Food categories	Variable	Function	Minimum	Mean	Maximum	% Consumers
Chips	Food intake	RiskExpon(0.0000429428;RiskShift(−0.0000000132662))	0	0.00004	+∞	10
	EFA content	RiskTriang(55.347;55.347;934.61)	55.35	348.43	+∞	
Cookies	Food intake	RiskExpon(0.0000392046;RiskShift(−0.0000000121414))	0	0.00004	+∞	12
	EFA content	RiskLoglogistic(53.82;85.797;3.7601)	53.82	150.48	+∞	
Dry nuts	Food intake	RiskExpon(0.0000301116;RiskShift(−0.0000000094246))	0	0.00003	+∞	5
	EFA content	RiskGamma(1.3566;393.79;RiskShift(152.76)	152.76	686.98	+∞	
French fries	Food intake	RiskExpon(0.00032186;RiskShift(−0.0000000993093))	0	0.00032	+∞	25
	EFA content	RiskExtvalue(98.855;50.892)	−∞	128.23	+∞	
Plant oils	Food intake	RiskExpon(0.00029172;RiskShift(−0.0000000900938))	0	0.00029	+∞	36
	EFA content	RiskExpon(380.31;RiskShift(19.814)	19.81	400.12	+∞	
Bacon	Food intake	RiskExpon(0.0000630029;RiskShift(−0.0000000195116))	0	0.0001	+∞	9
	EFA content	RiskTriang(7.2532;7.2532;210.85)	7.25	75.12	+∞	
Butter–margarine	Food intake	RiskInvgauss(0.0012506;0.0038708;RiskShift(−0.00011301))	−0.0001	0.00114	+∞	91
	EFA content	RiskInvgauss(331.75;577.04)	77.18	408.93	+∞	
Cheese	Food intake	RiskExpon(0.00045229;RiskShift(−0.000000139467))	0	0.00045	+∞	45
	EFA content	RiskExtvalue(248.63;105.24)	−∞	309.38	+∞	
Cooked meat	Food intake	RiskExpon(0.0000890779;RiskShift(−0.0000000275017))	0	0.00010	+∞	10
	EFA content	RiskExtvalue(9.0545;5.4844)	−∞	12.220	+∞	
Mayonnaise	Food intake	RiskGamma(2.4914;0.00063544;RiskShift(0.00031812))	0.0003	0.00190	+∞	44
	EFA content	RiskLognorm(159.21;240.26)	135.84	295.05	+∞	
Milk	Food intake	RiskExpon(0.0078497;RiskShift(−0.00000242049))	0	0.00785	+∞	64
	EFA content	RiskLogistic(36.963;11.249)	−∞	36.96	+∞	
Raw ham	Food intake	RiskExpon(0.0000362156;RiskShift(−0.0000000112192))	0	0.00004	+∞	11
	EFA content	RiskExpon(50.159;RiskShift(13.407)	13.41	63.57	+∞	
Cured minced raw meat	Food intake	RiskExpon(0.0000536806;RiskShift(−0.0000000166194))	0	0.00005	+∞	14
	EFA content	RiskLoglogistic(−23.764;211.55;3.7606)	−23.7600	214.55	+∞	
Smoked salmon	Food intake	RiskExpon(0.0000188984;RiskShift(−0.00000000591313))	0	0.00002	+∞	4
	EFA content	RiskInvgauss(66.628;139.396;RiskShift(2.4924)	2.4900	69.12	+∞	
Fresh and frozen salmon	Food intake	RiskExpon(0.0000838218;RiskShift(−0.0000000263756))	0	0.00008	+∞	4
	EFA content	RiskExtvalue(54.337;47.07)	−∞	81.5100	+∞	
Snack foods	Food intake	RiskGamma(0.94776;0.0016524)	0	0.00160	+∞	3
	EFA content	RiskLogistic(190.92;38.031)	−∞	190.9200	+∞	
Ready-to-eat meals	Food intake	RiskDiscrete(0.003.0.022.0.138.0.415.0.239;0.855.0.427.0.142.0.066.0.033.0.003)	0.0030	0.03800	0.4153	78
	EFA content	RiskLoglogistic(17.1;29.533;2.0843)	17.1000	61.7000	+∞	

Note: EFA, epoxy fatty acids methyl esters.

fitted to the best distributions, which were defined based on the lowest chi-square statistics and P-P plots attributed in @Risk software (Table 4). Before quantitative analysis using the fitted distributions, stability and reproducibility were tested, whereby three simulations with 50,000 iterations were made, and the results were found to be consistent and reproducible. Table 4 shows the best-fit distribution functions and the parameters describing them. The accompanying statistics that are required for the calculation (minimum, mean and maximum) are also shown. These are random values chosen by @Risk after considering the uncertainty in the two data inputs (concentration and consumption) to represent the minimum, mean and maximum values of the concentration of EFAs, and the consumption of the foods in question within the defined distributions. Results from the probabilistic estimates of the intake (mean, standard deviation, maximum, percentiles) resulting from the consumption by both consumers and the total population of each food category are presented in Table 5.

Food ranking per the percentage exceeding the TTC show that butter–margarine, mayonnaise and ready-to-eat meals had 100% of consumers above the TTC, which corresponded to 50% of the total population in butter–margarine and mayonnaise. Other foods in decreasing order of exceedance were cheese, snacks foods, plant oils, French fries, dry nuts, chips, cured minced raw meat, cookies, fresh and frozen salmon, bacon, raw cured ham, smoked salmon, and cooked meat. At P50, it can be concluded that the highest contribution to the intake of EFAs comes from mayonnaise, margarine–butter and the ready-to-eat meals, which are 0.4085, 0.3328 and 0.2997 mg kg^{-1} bw day^{-1} respectively. The probabilistic approach had a higher mean intake of ready-to-eat meals (2.52 mg kg^{-1} bw day^{-1}) by a magnitude of eight times compared with the median (P50) (0.3 mg kg^{-1} bw day^{-1}) because of the high positive skewness in the distributions.

Table 5. Estimated intakes (mg kg^{-1} bw day^{-1}) of EFAs from different foods using probabilistic approach for Belgian consumers and population.

Food categories		Mean	SD	P50	P75	P90	P97.5	P99.5	Maximum	% >TTC
Chips	Consumers	0.0150	0.0196	0.0081	0.0191	0.0377	0.0705	0.1113	0.3330	86
	Total population	0.0075	0.0155	0.0000	0.0081	0.0232	0.0528	0.0907	0.3095	43
Cookies	Consumers	0.0059	0.0065	0.0038	0.0079	0.0139	0.0233	0.0362	0.1002	76
	Total population	0.0029	0.0055	0.0000	0.0038	0.0092	0.0185	0.0304	0.1002	38
Dry nuts	Consumers	0.0207	0.0286	0.0112	0.0256	0.0499	0.0981	0.1686	0.5925	90
	Total population	0.0103	0.0228	0.0000	0.0113	0.0309	0.0717	0.1350	0.5925	45
French fries	Consumers	0.0413	0.0511	0.0243	0.0538	0.0989	0.1809	0.2966	0.8278	95
	Total population	0.0208	0.0415	0.0000	0.0246	0.0652	0.1384	0.2452	0.7068	48
Plant oils	Consumers	0.1165	0.1929	0.0483	0.1343	0.2989	0.6488	1.1498	4.9023	97
	Total population	0.0583	0.1500	0.0001	0.0481	0.1700	0.4553	0.9220	4.9023	48
Bacon	Consumers	0.0047	0.0064	0.0024	0.0060	0.0120	0.0227	0.0370	0.1117	62
	Total population	0.0024	0.0052	0.0000	0.0024	0.0074	0.0173	0.0313	0.1117	31
Butter	Consumers	0.4656	0.4460	0.3328	0.5751	0.9489	1.6584	2.7921	8.6917	100
	Total population	0.2337	0.3944	0.0370	0.3336	0.6588	1.2757	2.2471	8.6917	50
Cheese	Consumers	0.1395	0.1642	0.0866	0.1844	0.3292	0.5826	0.9543	2.6107	99
	Total population	0.0702	0.1367	0.0002	0.0871	0.2176	0.4498	0.7973	2.4784	50
Cooked meat	Consumers	0.0011	0.0014	0.0006	0.0014	0.0027	0.0050	0.0083	0.0263	23
	Total population	0.0005	0.0011	0.0000	0.0006	0.0017	0.0038	0.0069	0.0263	12
Mayonnaise	Consumers	0.5616	0.6026	0.4085	0.6527	1.0491	1.9647	3.7183	22.1083	100
	Total population	0.2821	0.5122	0.0750	0.4076	0.7404	1.4665	2.9125	18.3956	50
Milk	Consumers	0.2899	0.3663	0.1712	0.3917	0.7187	1.3009	2.0624	5.8169	96
	Total population	0.1438	0.2929	0.0000	0.1724	0.4670	0.9877	1.6630	4.2087	48
Raw ham	Consumers	0.0023	0.0034	0.0011	0.0028	0.0057	0.0115	0.0211	0.0534	42
	Total population	0.0011	0.0027	0.0000	0.0012	0.0033	0.0084	0.0168	0.0534	21
Cured minced raw meat	Consumers	0.0115	0.0151	0.0067	0.0146	0.0272	0.0513	0.0872	0.4979	84
	Total population	0.0057	0.0121	0.0000	0.0067	0.0176	0.0382	0.0710	0.2834	42
Smoked salmon	Consumers	0.0013	0.0018	0.0007	0.0016	0.0031	0.0060	0.0108	0.0306	27
	Total population	0.0007	0.0014	0.0000	0.0007	0.0020	0.0045	0.0085	0.0306	14
Fresh and frozen salmon	Consumers	0.0068	0.0098	0.0035	0.0087	0.0174	0.0338	0.0563	0.1811	69
	Total population	0.0034	0.0078	0.0000	0.0034	0.0106	0.0251	0.0471	0.1811	34
Snack foods	Consumers	0.2982	0.3411	0.1869	0.4032	0.7171	1.2255	1.8712	6.5076	99
	Total population	0.1478	0.2794	0.0000	0.1854	0.4713	0.9679	1.5586	4.0098	49
Ready-to-eat meals[a]	Consumers	2.5223	10.3656	0.2997	1.3978	7.0671	17.4940	38.6068	961.2216	100

Notes: [a]The population was obtained from the survey conducted by Daelman et al. (2013).
TTC, threshold of toxicological concern.

Both the deterministic and probabilistic approaches had nearly the same mean estimation of the intake, except in the snack foods where the deterministic approach overestimated the intake by 3%. The P99.5 estimate of the deterministic approach had higher exposures by a magnitude of 2–7 times in other foods; however, the estimate for cooked meat was 21 times higher (Tables 3 and 5). Similar observations were made by Papastergiadis et al. (2014) when studying the intake of aldehydes in similar food categories, where the deterministic approach had a similar mean estimation and it tended to overestimate the intake at both P99.5 and at maximum intake. This observation mainly depends on the type of distribution that is fitted to the data. Highly skewed distributions tend to have higher means and P99.5 estimates (Papastergiadis et al. 2014).

Risk characterisation

Due to lack of toxicological data on EFAs, the TTC principle was applied. Based on the Cramer decision tree, EFAs were grouped in class III and, therefore, a TTC level of exposure of 0.0015 mg kg^{-1} bw day^{-1} was considered when evaluating the intake take data obtained from the probabilistic analysis. The average body weight was considered to be 60 kg (Kroes et al. 2005). Based on the probabilistic analysis of the consumption and the contamination data, it can be suggested that consumers of the studied food categories may be at risk, since exposure was far above the defined TTC. The exception was cooked meat, smoked salmon and raw cured ham, which were below the TTC with P50 intakes of 0.0006, 0.0007 and 0.0011 mg kg^{-1} bw day^{-1} respectively. This corresponded to 77%, 73% and 58% of consumers of these specific food groups exposed to EFAs at levels below the TTC of 1.5 mg kg^{-1} bw day^{-1} respectively. The rest of the consumers of the other foods were exposed to high levels, which were up to 200 times far above the TTC with 100% of consumers of mayonnaise, butter–margarine and ready-to-eat meals at risk. Thus, a potential risk may occur for this portion of consumers frequently consuming these specific food categories.

Uncertainty evaluation of the exposure assessment

There are always uncertainties related to exposure assessments that should be considered for the interpretation of the results. The BNFCS used in this study was conducted in 2004, and it is known that eating habits might change over time. Therefore, it is not clear if the dietary intake of the Belgian population has significantly changed. This might lead to some uncertainties during interpretation of the results. However, a new evaluation study was conducted in 2014 by the public health authority, although the results are not yet available. A possible under- or overestimation of the consumption of the studied food groups resulting from misreporting during consumption data collection (e.g., inaccuracies on consumed quantities reported, foods reported in the wrong food groups) could be some of the limiting factors in the precision of the estimated intakes for the EFAs. Despite this, 24-h recall is usually the best recommended method used to estimate dietary food intake in large consumption surveys in Europe (de Boer et al. 2011). For chilled cooked meals, the consumption data do not represent the total population residing in Belgium, thus deviations could be expected from the national consumption of these products. The current calculations and interpretations are performed for the individual food categories; no inference can be made for the total exposure resulting from the consumption of other foods by Belgian consumers.

Furthermore, the risk from exposure to EFAs in this study is evaluated as a total and not as individual isomers. Such an exposure is cumulative in that it considers the combined toxicological effect of a group of chemicals with an assumed common mechanism of toxicity (Gosens et al. 2014). Compounds with a common toxicity mechanism are advised to be put into cumulative assessment groups (CAGs) (Kennedy et al. 2015). In view of their structural differences in terms of unsaturation, it may be incorrect to do this since reactivity differences for each isomer may occur. This may imply that a combined estimation of all the EFAs could be an underestimation of the possible risk they can cause depending on the behaviour of an individual isomer in the body. However, on the other hand, it is also possible that a combined exposure of all 12 isomers would lead to an

overestimation of the risk that they may pose and lead to an erroneous conclusion that many consumers are at risk as predicted from such a study which does not consider individual isomer exposure.

Conclusions

This is the first study concerning the exposure assessment of a population to EFAs through the consumption of specific foods. The exposure related to the consumption of the specific food categories and the resulting potential risk have been reported. Results indicate that consumers of the studied food categories are exposed to high levels of EFAs. Based on the TTC principle as a preliminary risk characterisation approach, results suggest that a risk could exist due to the consumption of these foods for the vast majority of consumers. Since the TTC is a preliminary approach, it is recommended that extensive toxicity testing and safety evaluation of EFAs be done to characterise fully the risk involved, especially in plant-based foods.

An aggregate exposure approach for lipid oxidation compounds to assess the potential risk should be performed. In such a study, EFAs should be combined with those oxidation products (MDA, HNE and HHE) presented by Papastergiadis et al. (2014) to obtain a total picture of the toxic oxidation compounds to which consumers may be exposed. It is also known that EFAs once formed cannot be removed, and highly nutritious foods like nuts have been shown to contribute significantly to the intake of EFAs. Therefore, it should be advised that a reduction of the intake of EFAs through the prevention of lipid oxidation is needed. This can be achieved by taking precautions to prevent lipid oxidation in foods, especially during processing and storage, which may lower the consumption of such oxidised foods.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors acknowledge Ghent University through the Concerted Research Actions (GOA) project for funding this research. Antonios Papastergiadis was funded by the Federal Public Service of Health, Food Chain Safety and Environment [grant number RT/10 TOXAL]. Kshitij Shrestha obtained a grant from the Special Research Fund (BOF) scholarship from Ghent University. Rubel Mozumder was funded by the VLIR-UOS.

References

Cramer GM, Ford RA, Hall RL. 1978. Estimation of toxic hazard – a decision tree approach. Food Cosmet Toxicol. 16:255–276.

Daelman J, Jacxsens L, Membré J-M, Sas B, Devlieghere F, Uyttendaele M. 2013. Behaviour of Belgian consumers, related to the consumption, storage and preparation of cooked chilled foods. Food Control. 34:681–690.

de Boer EJ, Slimani N, van't Veer P, Boeing H, Feinberg M, Leclercq C, Trolle E, Amiano P, Andersen LF, Freisling H. 2011. The European food consumption validation project: conclusions and recommendations. Eur J Clin Nutr. 65: S102–S107.

Daelman J, Jacxsens L, Membré J-M, Sas B, Devlieghere F, Uyttendaele M. 2013. Behaviour of belgian consumers, related to the consumption, storage and preparation of cooked chilled foods. Food Control. 34:681–690.

De Vriese S, De Backer G, De Henauw S, Huybrechts I, Kornitzer K, Levêque A, Moreau MVan Oyen H. 2005. The belgian food consumption survey: aims, design and methods. Archives Of Public Health. 63:1–16.

Fankhauser-Noti A, Fiselier K, Biedermann-Brem S, Grob K. 2006. Assessment of epoxidized soy bean oil (ESBO) migrating into foods: comparison with ESBO-like epoxy fatty acids in our normal diet. Food Chem Toxicol. 44:1279–1286.

Frankel EN. 2005. Lipid oxidation. 2nd ed. Cambridge, UK: The Oil Press.

Gosens I, Delmaar CJ, ter Burg W, de Heer C, Schuur AG. 2014. Aggregate exposure approaches for parabens in personal care products: a case assessment for children between 0 and 3 years old. J Expo Sci Environ Epidemiol. 24:208–214.

Greene JF, Newman JW, Williamson KC, Hammock BD. 2000. Toxicity of epoxy fatty acids and related compounds to cells expressing human soluble epoxide hydrolase. Chem Res Toxicol. Apr;13:217–226.

Greene JF, Williamson KC, Newman JW, Morisseau C, Hammock BD. 2000. Metabolism of monoepoxides of methyl linoleate: bioactivation and detoxification. Arch Biochem Biophys. 376:420–432.

Gunstone FD, Harwood JL, Dijkstra AJ. 2012. The lipid handbook with CD-ROM. 3rd ed. Boca Raton (FL): CRC Press.

Gunstone FD, Jacobsberg FR. 1972. Fatty acids, part 35 the preparation and properties of the complete series of methyl epoxyoctadecanoates. Chem Phys Lipids. 9:26–34.

Gunstone FD, Schuler HR. 1975. Fatty acids, part 45 epoxyoctadecenoates, dihydroxyoctadecenoates, and

diepoxyoctadecanoates: preparation, chromatographic properties, and reaction with boron trifluoride etherate. Chem Phys Lipids. 15:174–188.

Harttig U, Haubrock J, Knueppel S, Boeing H, Consortium EFCOVAL. 2011. The MSM program: web-based statistics package for estimating usual dietary intake using the multiple source method. Eur J Clin Nutr. Jul;65:S87–S91.

Harwood JL, Dijkstra AJ, Gunstone FD. 2007. The lipid handbook. Boca Raton (FL): CRC Press.

Haubrock J, Noethlings U, Volatier J-L, Dekkers A, Ocke M, Harttig U, Illner A-K, Knueppel S, Andersen LF, Boeing H. 2011. Estimating usual food intake distributions by using the multiple source method in the EPIC-potsdam calibration study. J Nutr. May;141:914–920.

Hennes EC. 2012. An overview of values for the threshold of toxicological concern. Toxicol Lett. 211:296–303.

Huybrechts I, Matthys C, Vereecken C, Maes L, Temme EHM, Van Oyen H, De Backer G, De Henauw S. 2008. Food intakes by preschool children in Flanders compared with dietary guidelines. Int J Environ Res Public Health. 5:243–257.

Institute of Human Nutrition Potsdam-Rehbrücke (DIfE). 2013. The multiple source method (MSM). https://msm.dife.de

Kennedy MC, Glass CR, Bokkers B, Hart AD, Hamey PY, Kruisselbrink JW, de Boer WJ, van der Voet H, Garthwaite DG, van Klaveren JD. 2015. A European model and case studies for aggregate exposure assessment of pesticides. Food Chem Toxicol. 79:32–44.

Kroes R, Kleiner J, Renwick A. 2005. The threshold of toxicological concern concept in risk assessment. Toxicological Sci. Aug 1;86:226–230.

Kroes R, Müller D, Lambe J, Löwik MRH, van Klaveren J, Kleiner J, Massey R, Mayer S, Urieta I, Verger P, et al. 2002. Assessment of intake from the diet. Food Chem Toxicol. 40:327–385.

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, et al. 2004. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem Toxicol. Jan;42:65–83.

Medeiros Vinci R, Jacxsens L, Van Loco J, Matsiko E, Lachat C, de Schaetzen T, Canfyn M, van Overmeire I, Kolsteren P, De Meulenaer B. 2012. Assessment of human exposure to benzene through foods from the Belgian market. Chemosphere. 88:1001–1007.

Mubiru E, Shrestha K, Papastergiadis A, De Meulenaer B. 2013. Improved gas chromatography-flame ionization detector analytical method for the analysis of epoxy fatty acids. J Chromatogr. 1318:217–225.

Mubiru E, Shrestha K, Papastergiadis A, De Meulenaer B. 2014. Development and validation of a gas chromatography–flame ionization detection method for the determination of epoxy fatty acids in food matrices. J Agric Food Chem. Apr 2;62:2982–2988.

Papastergiadis A, Fatouh A, Jacxsens L, Lachat C, Shrestha K, Daelman J, Kolsteren P, Langenhove H, De Meulenaer B. 2014. Exposure assessment of Malondialdehyde, 4-Hydroxy-2-(E)-Nonenal and 4-Hydroxy-2-(E)-Hexenal through specific foods available in Belgium. Food Chem Toxicol. 73:51–58.

Picot, C., & Roudot, A. C. (2012). A practical example of risk assessment: risk assessment to phycotoxins in a recreational shellfish harvester's subpopulation. INTECH. p. 1–19.z

Rennen MAJ, Koster S, Krul CAM, Houben GF. 2011. Application of the threshold of toxicological concern (TTC) concept to the safety assessment of chemically complex food matrices. Food Chem Toxicol. 49:933–940.

Schaich KM. 2005. Lipid oxidation: theoretical aspects. In: Shahidi F, editor. Bailey's industrial oil and fat products. 6th ed. Hoboken (NJ): Wiley Interscience, A John Wiley & Sons, Inc., Publication; p. 269–355.

Spitzer V. 1999. Screening analysis of unknown seed oils. Lipid /Fett. 101:2–19.

Steele R. 2004. Understanding and measuring the shelf-life of food. Cambridge, UK: Woodhead Publishing.

Swern D. 1955. Oxygenated fatty acids. Prog Chem Fats Other Lipids. 3:213–239.

Temme E, Huybrechts I, Vandevijvere S, De Henauw S, Leveque A, Kornitzer M, De Backer G, Van Oyen H. 2010. Energy and macronutrient intakes in Belgium: results from the first national food consumption survey. Br J Nutr. Jul 28;103:1823–1829.

USFDA. 1995. Food additives: threshold of regulation for substances used in food-contact articles (final rule). Fed Regist. 60:36582–36596.

Vandevijvere S, De Vriese S, Huybrechts I, Moreau M, Temme E, De Henauw S, De Backer G, Kornitzer M, Leveque A, Van Oyen H. 2009. The gap between food-based dietary guidelines and usual food consumption in Belgium, 2004. Public Health Nutr. 12:423–431.

Vose D. 2008. Risk analysis: a quantitative guide West Sussex, PO19 8SQ. England: John Wiley & Sons.

Vromman V, Waegeneers N, Cornelis C, De Boosere I, Van Holderbeke M, Vinkx C, Smolders E, Huyghebaert A, Pussemier L. 2010. Dietary cadmium intake by the Belgian adult population. Food Additives Contam Part A. 27:1665–1673.

Wilson R, Fernie CE, Scrimgeour CM, Lyall K, Smyth L, Riemersma RA. 2002. Dietary epoxy fatty acids are absorbed in healthy women. Eur J Clin Invest. 32:79–83.

Woodgate SL, van der Veen JT. 2014. 21 Fats and Oils–Animal Based. In: Food Processing: Principles and Applications. 2nd ed. Hopoken (NJ) : Wiley.

Zheng J, Plopper CG, Lakritz J, Storms DH, Hammock BD. 2001. Leukotoxin-diol – A putative toxic mediator involved in acute respiratory distress syndrome. Am J Respir Cell Mol Biol. Oct;25:434–438.