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Identification of chemical mixtures to which women are exposed through the diet: Results from the French E3N cohort

Francesca Romana Mancini [a,*], Pauline Frenoy [a], Thibault Fiolet [a], Guy Fagherazzi [b], Amélie Crépet [c]

[a] Université Paris-Saclay, UVSQ, Inserm, Gustave Roussy, "Exposome and Heredity" Team, CESP UMR1018, 94805 Villejuif, France

[b] Deep Digital Phenotyping Research Unit, Department of Population Health, Luxembourg Institute of Health, 1A-B, Rue Thomas Edison, L-1445 Strassen, Luxembourg

[c] French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Risk Assessment Department, Methodology and Survey Unit, 94701 Maisons-Alfort, France

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ABSTRACT

Due to the large number of chemical food contaminants, consumers are exposed simultaneously to a wide range of chemicals which can interact and have a negative impact on health. Nevertheless, due to the multitude of possible chemical combinations it is unrealistic to test all combined toxicological effects. It is therefore essential to identify the most relevant mixtures to which the population is exposed through the diet and investigate their impact on heath.

The present study aims to identify and describe the main chemical mixtures to which women enrolled in the E3N study, a large French prospective cohort, are chronically exposed through the diet.

74 522 women who had answered a validated semi-quantitative food frequency questionnaire in 1993, were included in the present study. Dietary exposure to chemical contaminates was estimated based on the food contamination measured in 186 core food in France collected between 2007 and 2009 by the French agency for food, environment and occupational health, and safety (ANSES) in the framework of the second French total diet study (2TDS). The sparse non-negative matrix under-approximation (SNMU) was used to identify mixtures of chemical substances. A k-means clustering classification of the whole study population was then performed to define clusters with similar co-exposure profiles.

Overall, 8 mixtures which explained 83% of the total variance, were retained. The first mixture, entitled "Minerals, inorganic contaminants, and furans", explained the highest proportion of the total variance (38%), and was correlated in particular with the consumption of "Offal" (rho = 0.22), "Vegetables except roots" (rho = 0.20), and "Eggs" (rho = 0.19). The other seven mixtures explained between 17% and 1% of the variance. Finally, 5 clusters were identified based on the adherence to the 8 mixtures.

This study, being the largest ever conducted to identify dietary exposure to chemical mixtures, represents a concrete attempt to prioritize mixtures for which it is essential to investigate combined health effects based on exposure.

1. Introduction

Due to the large number of chemicals found in the environment, populations worldwide are exposed to a wide range of chemicals which can interact and have health impacts (Bopp et al., 2018). The joint action of different chemical components with similar or dissimilar modes of action can result in additive, synergistic or antagonistic toxicological effects (Kamo and Yokomizo, 2015; Hernández et al., 2017; Chen et al., 2015). To date most toxicological, epidemiological and risk assessment studies investigate one substance at a time. However, not considering mixture effects can potentially result in a risk underestimation or overestimation. This approach is mainly due to the multitude of possible chemical combinations for which it is unrealistic to test combined toxicological effects. As it is not feasible to test every conceivable combination of agents, guidelines most often recommend to group substances from a same chemical family or that share the same mode of

* Corresponding author at: Inserm U1018, Centre for Research in Epidemiology and Population Health (CESP), "Exposome and Heredity" Team, Gustave Roussy, 114 rue Edouard Vaillant, F-94805 Villejuif, France.

E-mail address: Francesca.mancini@gustaveroussy.fr (F.R. Mancini).

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action due to the fact that for combinations of chemicals that act on the same molecular target, there is clear evidence that mixture effects can arise (EFSA, 2007; Scientific Opinion of the Panel on Plant Protection Products and their Residues, 2008; International Programme on Chemical Safety & Inter-Organization Programme for the Sound Management of Chemicals, 2009). Nevertheless there is also good evidence that combinations composed of chemicals with diverse modes of action but similar effects may induce mixture effects (Hadrup, 2014). Thus, the question of which substances must be assessed in combination remains a major challenge. As diet is one of major sources of exposure for a large range of chemicals, it is essential to identify the most relevant mixtures to which the population is exposed through the diet in order to investigate their impact on health (Traoré et al., 2016; Kopp et al., 2018).

In toxicology studies, chemicals' toxicity is typically assessed at high doses that are way above the levels of exposure in the general population. Nevertheless it has been suggested that for several substances low dose effects are possible, meaning that biological changes may be observed in the background exposure range in the general population or at doses lower than those generally used in standard toxicity assessment tests (Birnbaum, 2012). It is thus crucial to quantify the levels to which the general population is exposed in real life in order to consider also potential low-dose effects when investigating exposure to chemical mixtures.

The many potentially toxic substances in the environment may contaminate food consumed by people. Food contaminants, including inorganic and organic substances, may originate from a wide range of sources and can be of natural or anthropogenic origin and the diet can represent a major pathway of exposure for the general population (Thompson and Darwish, 2019; Gibb et al., 2015).

The present study aims to identify and describe the main chemical mixtures to which women enrolled in the large E3N prospective cohort are chronically exposed through the diet.

2. Materials and methods

2.1. E3N cohort

The E3N (Etude Epidémiologique auprès de femmes de l'Education Nationale) study is a French prospective cohort set up in metropolitan France in 1990 (Clavel-Chapelon, 2015). E3N includes 98 995 women born between 1925 and 1950 and affiliated to the French national health insurance plan for teachers and coworkers of the national education system, the Mutuelle Générale de l'Education Nationale (MGEN), at inclusion. Women were enrolled in the cohort through a self-administered questionnaire, and were followed by self-administered questionnaires on health conditions, lifestyle, diet, treatments, mental health status, etc. sent every two or three years. Starting in 2004, information on drug reimbursements by the MGEN was also collected. A more detailed description of the E3N cohort has been provided elsewhere (Clavel-Chapelon et al., 1997).

Average response rate at each follow-up questionnaire is of about 83%, and the overall loss to follow-up since 1990 is 3%. All participants in the cohort provided an informed consent, and the French National Commission for Computerized Data and Individual (CNIL) reviewed and approved the study.

Only E3N participants who had completed the dietary questionnaire sent in 1993 (n = 74 522) were included in the present study. All women with extreme energy intake values (i.e. the 1st and 99th percentiles of the energy intake over energy requirement distribution in the population) (n = 1491) were excluded so that finally 73 031 women were included in this study.

2.2. Dietary exposure assessment

The usual food consumption over the past year was estimated through a validated 208-item semi-quantitative food frequency questionnaire sent in 1993. The validity and reproducibility of the dietary questionnaire have been previously described (van Liere et al., 1997).

Data on food contamination were obtained from the 2nd French Total Diet Study (TDS2) published by the French agency for food, environment and occupational health, and safety (ANSES) (French agency for food, environment and occupational health & safety (ANSES), 2011; French agency for food, environment and occupational health & safety (ANSES), 2011). In short, a total of 20 280 different food products were collected between June 2007 and January 2009 in eight French regions, reaching 1352 composite samples of foods prepared as consumed and analyzed to measure the concentrations of more than four hundreds contaminants in 186 core foods (Sirot et al., 2009). The samples were prepared to avoid possible variations in contaminant concentrations due to food preparation and cooking process (e.g. peeling, washing, baking, or frying). Subsequently, the E3N databases on food consumption and the ANSES database on food contaminants concentrations have been merged as described in detail elsewhere (Mancini et al., 2020).

For the present study, all values below the limit of detection were replaced by 0 and all the values below the limit of quantification were replaced by the limit of detection when it was available, by 0 otherwise.

For each woman and substance included in the study, the individual chronic dietary exposure was estimated in summing exposure from each consumed food item as follows:

$$e_a^{(p)} = \frac{\sum_{f=1}^{\mu} q_{af} \times c_{fp}}{bw_a}$$

where $e_a^{(p)}$ represents the exposure of the individual a to the substance p, q_{af} is the quantity of food f consumed by individual a (in g per day), c_{fp} is the contamination level for substance p in food f (in ng of substance per g of food), and bw_a is the body weight of individual a (in kg).

Substances with exposure equal to zero for all individuals were excluded from the analysis. These substances were:

- 264 active pesticide residues;
- 4 perfluorinated compounds (PFAS): PFHpS, PFDS, PFBA, PFPA;
- 8 mycotoxins: Aflatoxins (AFs) B2, G1, M1, verrucarol, monoacetoxyscirpenol, beta zearalanol, beta zearalenol, deepoxy derivative of DON;

In addition we excluded data concerning food additives due to the fact that the quantity of food additives used in the same food product by different brands may vary greatly. Consumers tend to develop "brand-loyalty" and buy specific food products always of the same brand. Among data available in the E3N, no information concerning the brand of the food products consumed is available. This missing information made it not possible to take into account the "brand-loyalty" introducing the risk of underestimating (or overestimating) the average individual exposure to food additives.

Finally, the following 197 substances were included in the present study and formed the exposure matrix E (197 × 73 031):

- 16 inorganic contaminants: aluminium (Al), antimoine (Sb), argent (Ag), arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), tin (Sn), gallium (Ga), germanium (Ge), mercury (Hg), nickel (Ni), lead (Pb), strontium (Sr), tellure (Te), vanadium (V);
- 12 minerals: calcium (Ca), chrome (Cr), cuivre (Cu), fer (Fe), lithium (Li), magnésium (Mg), manganèse (Mn), molybdène (Mo), potassium (K), selenium (Se), sodium (Na), zinc (Zn);
- 17 congeners of polychlorinated dibenzo-p-dioxins (or dioxins) and polychlorinated dibenzofurans (or furans) (TCDD-2378, PCDD-12378, HCDD-123478, HCDD-123678, HCDD-123789, HCDD-1234678, OCDD, TCDF-2378, PCDF-12378, PCDF-23478, HCDF-

123478, HCDF-123678, HCDF-234678, HCDF-123789, HCDF-1234678, HCDF-1234789, OCDF);

- 18 polychlorinated biphenyls (PCB), of which 12 congeners 'dioxin-like' (PCB-DL 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189) and 6 congeners 'non dioxin-like' (PCB-NDL 28, 52, 101, 138, 153, 180);
- 12 PFAS (PFDA, PFDoA, PFHpA, PFHxA, PFNA, PFOA, PFTeDA, PFTrDA, PFUnA, PFBS, PFHxS, PFOS);
- 14 brominated flame retardants (BFRs): eigth polybrominated diphenyl ether congeners (PBDE-28, 47, 99, 100, 153, 154, 183, 209), three polybrominated biphenyl congeners (PBB-52, 11, 153) and three hexabromocyclododecane congeners (HBCD-alpha, beta, gamma);
- 18 mycotoxins: fumonisins B1 and B2 (FB1, FB2), aflatoxins (AFs) B1 and G2, ochratoxin A, B (OTA, OTB) and patulin (Pat), trichothecenes from group A, including T2-toxin, HT2-toxin, diacetoxyscirpenol (DAS), and from group B, including nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (DON3), 15-acetyldeoxynivalenol (DON15),and fusarenon X (FusX), zearalenone (Zea) and its metabolites: alpha-zearalanol and alpha-zearalenol;
- 11 phytoestrogens: biochanin A, coumestrol, daidzein, enterolactone, equol, formononetin, genistein, glycitein, matairesinol, resveratrol, and secoisolariciresinol;
- 58 active pesticide residues;
- Acrylamide
- 20 PAHs: anthrancene (AN), benzo [a]anthracene (BaA), benzo [a] pyrene (BaP), benzo [b] fluoranthene (BbF), benzo [c]fluorine (BcFL), benzo [g,h,i]perylene (BghiP), benzo [j]fluoranthene (BjF), benzo [k]fluoranthene (BkF), chrysene (CHR), cyclopenta (c,d)pyrene (CPP), dibenzo [a,h]anthracene (DBahA), dibenzo [a,e]pyrene (DbaeP), dibenzo [a,h]pyrene (DbahP), dibenzo [a,i]pyrene (DbaiP), dibenzo [a,l]pyrene (DbalP), fluoranthene (FA), indeno [1,2,3-cd] pyrene (IP), 5-methylchrysene (MCH), phenanthrene (PHE) and pyrene (PY);

2.3. Chemical mixture identification

The sparse non-negative matrix under-approximation (SNMU) was used to identify mixtures of chemical substances (Gillis and Glineur, 2009; Gillis and Plemmons, 2013; Traoré et al., 2018). This method is derived from non-negative matrix factorization (NMF) (Lee and Seung, 2001), which has been previously used by other authors (Traoré et al., 2016; Bechaux et al., 2013). SNMU is a method of reduction of dimensions applicable to a non-negative matrix of real numbers, therefore it is adapted to exposure data. SNMU consists in factorization of the exposure matrix E in two matrices U and V, such as E = U*V+Ɛ. The matrix U is the matrix of mixtures and their substance composition. Each column represents a mixture, and each line a substance. A weight is given to each substance composing a mixture, which is equal to 0 if the substance is not contributing to the mixture. The matrix V is the matrix of individual exposure to the mixtures. Each column represents an individual, and each line a mixture. For each couple individual/mixture, a statistical weight is attributed representing the strength of the adherence of an individual to a mixture, and thus can be interpreted as the level of exposure of the individual to the mixture.

The optimization method of the NMF consists in finding by convergence the best couple UV which minimizes the difference Ɛ between E and UV ($\|E - UV\|^2$ *such that* $U \geq 0, \quad V \geq 0$ *and* $UV \leq E$) (Lee and Seung, 2001). Gillis and Glineur (2010) proposed the NMU in adding a new constraint to this optimization problem to make it possible to identify exposure systems one by one (Gillis and Glineur, 2009). Then, Gillis and Plemmons (Gillis and Plemmons, 2013) proposed the SNMU in adding a sparsity constraint to reinforce the separation of the substances contributing weakly from those contributing strongly to mixtures (Gillis and Plemmons, 2013). The sparsity constraint was defined for each mixture using a sparsity parameter with bounds: the value of the sparsity parameter decreases when the lower bound is reached, and increases when the upper bound is reached at each iteration (Gillis and Plemmons, 2013). The value of the parameter and its bounds were chosen after performing several tests as done in Traoré et al. (2018).

Strong constraints of sparsity applied to the first mixtures, which explain the majority of the variance, made it possible to force the latter to be parsimonious and not to include all the substances. Thus, the fixed sparsity parameters for the first four mixtures were 0.95, 0.8, 0.65, 0.5, respectively. For the remaining mixtures the sparsity was fixed at 0.4. The bounds were chosen wide, because after various tests this resulted in more parsimonious mixtures. Thus, the sparsity lower and upper bounds conserved were respectively 0.1 and 1. The maximal number of mixtures was fixed as 20, which seems a reasonable starting number of mixture regarding the number of studied substances. The SNMU method was applied to each number i of mixtures less than or equal to the maximum number of mixtures set ($1 \leq i \leq 20$). Three criteria were used to choose the final number of mixtures k_{opti}. As previously used by other authors (Bechaux et al., 2013; Zetlaoui et al., 2011; Sy et al., 2013), the Root-Mean-Square Error RMSE(i) were calculated for each number of mixture i. Then, a Ratio Difference RD(i) was calculated for each i as follow:

$$RD(i) = \frac{RMSE(i)}{RMSE(i-1)} - \frac{RMSE(i-1)}{RMSE(i-2)} \quad for \quad i > 2 \quad and \quad RD(2) = 0$$

First, the number of mixtures with the highest RD were considered as good candidates. Second, the overall percentage of variance explained by the candidate number of mixtures was calculated. Finally, the interpretability and relevance of the identified mixtures was investigated. The SNMU algorithm coded on R software was used for the analyses.

Partial Spearman rank correlation coefficients between weights of adherence to each mixtures retained (matrix V) and dietary consumption of the 24 main food groups, adjusted on other mixtures retained, were calculated.

2.4. Individuals clusters identification

A cluster classification of the whole population was performed from the matrix V to identify groups of individuals with similar weight of adherence to mixtures. We used the k-means clustering method implemented in the *FASTCLUS* procedure on SAS, with different maximum numbers of clusters tested (3, 4 and 5, based on the number of mixtures finally retained). Then, the final number of cluster retained was chosen on the basis of two criteria: the number of subjects in each cluster (clusters with not too disproportionate size were preferred), and the discrimination of clusters with regard to mean adherence to mixtures compared to the general population.

For each cluster, principal characteristics (age, BMI, physical activity, smoking status and education level) were described (mean for quantitative variables, proportion for categorical variables), and compared to the general population using compliance tests (*t*-test for quantitative variables, chisq test for categorical variables). The average weight of adherence to each mixture for all clusters was calculated and compared to those in the whole population using compliance t-tests.

For each cluster, the relative contribution of each mixture (in percentages) was calculated as follows:

$$c_{ij} = \frac{\mu_{ij}}{\sum_{i=1}^{k_{opti}} \mu_{ij}} * 100$$

were c_{ij} represents the contribution of mixture i to cluster j, μ_{ij} represents the average weight of adherence to mixture i among individuals of cluster j, and k_{opti} is the final number of mixtures selected.

3. Results

3.1. Description of the main mixtures to which E3N women are exposed through the diet

Women included in the present study were on average 52.9 ± 6.7 years old on average, with a BMI of 22.9 ± 3.2 kg/m^2, fairly active (46.4 ± 50.1 MET.hours/week), 46% had never smoked and 35.9% had received over 14 years of school education.

Based on the three criteria presented in section 2.3, we decided to retain 8 mixtures which explained overall 83% of the variance. In particular, the first mixture explained 38% of the variance, whereas the second, third, fourth, fifth, sixth, seventh, and eighth mixtures explained 17%, 6%, 5%, 3%, 8%, 5%, and 1% of the variance, respectively (Table 1). Table 1 presents the contribution (%) obtained from the weights of the matrix U of the 25 substances that contribute the most to

Table 1
Description of the main mixtures identified from E3N cohort (n = 73 031).

Mixture 1 Minerals, inorganic contaminants, and furans		Mixture 2 PCB, furan, and BFR		Mixture 3 Mycotoxins, pesticides, and PAH		Mixture 4 PCB, BFR, furans, Hg, and PFAS	
substance	%contrib	substance	%contrib	substance	%contrib	substance	%contrib
Cr	3.61%	PCB 118	4.34%	DON	9.19%	PCB 101	8.62%
K	3.42%	PCB 189	4.27%	Pirimiphos methyl	8.97%	BDE154	8.58%
Pb	3.11%	PCB 157	4.27%	HT2	8.2%	BB153	7.55%
Fe	3.08%	PCB 138	4.23%	OTA	7.99%	BDE28	7.45%
Zn	3.04%	PCB 114	4.21%	BghiP	7.78%	TCDF 2378	7.42%
Cd	2.86%	PCB 153	4.19%	CPP	7.32%	BDE100	7.12%
HCDF 1234789	2.83%	PCB 167	4.11%	ZEA	6.45%	Hg	6.97%
HCDF 1234678	2.81%	PCB 105	4.09%	Piperonyl butoxide	5.87%	PFOS	6.36%
OCDF	2.79%	PCDF 12378	3.78%	BjF	5.06%	PFUnA	5.93%
Ba	2.78%	PCB 169	3.68%	BbF	5.02%	PFTrDA	5.9%
HCDF 123478	2.78%	HBCD gamma	3.41%	BkF	4.71%	BB52	5.43%
Mo	2.71%	HBCD beta	3.4%	BaP	4.03%	PCB 156	4.67%
HCDF 234678	2.59%	HBCD alpha	3.39%	DBahA	3.67%	BB101	4.32%
Ni	2.59%	PCB 77	3.33%	Niv	3.58%	Ag	2.53%
HCDF 123678	2.55%	PCB 52	3.02%	acrylamide	2.44%	PFOA	2.39%
FA	2.54%	PCB 123	2.82%	FB1	2.21%	Daidzeine	2.13%
PCDF 23478	2.47%	BDE183	2.69%	IP	2.05%	Li	1.34%
V	2.46%	CHR	2.67%	Chlorpropham	1.91%	Se	1.33%
Na	2.44%	AN	2.58%	Li	1.00%	Genisteine	1.19%
HCDD 1234678	2.38%	BcFL	2.57%	T2	0.99%	Coumestrol	0.93%
N = 45 Sp = 0.77 %Ve = 38.17%		N = 41 Sp = 0.79 %Ve = 17.13		N = 26 Sp = 0.87 %Ve = 5.50		N = 25 Sp = 0.87 %Ve = 4.68	

Mixture 5 Pesticides (1)		Mixture 6 Pesticides (2)		Mixture 7 Pesticides (3)		Mixture 8 Mycotoxins and PAH	
substance	%contrib	substance	%contrib	substance	%contrib	substance	%contrib
Iprodione	12.76%	Bupirimate	5.5%	Fenhexamid	5.69%	OTB	12.16%
Lambda Cyhalothrin	12.09%	Phosmet	4.8%	Thiabendazole	5.6%	alpha ZAL	11.7%
Fludioxonyl	12.07%	Carbendazim	4.7%	Azoxystrobin	5.33%	alpha ZOL	11.7%
Sulfur	11.23%	Kresoxim methyl	4.63%	Diphenylamine	5.16%	DAS	11.7%
Cyprodinyl	10.72%	Vinclozolin	4.04%	Propargite	5.15%	FusX	11.7%
Procymidone	8.43%	Chlorpyrifos methyl	3.96%	Chlorpyrifos ethyl	4.9%	3 Ac DON	11.7%
Boscalid	7.23%	Quinoxyfen	3.83%	Acrinathrin	4.78%	T2	10.25%
PFOA	3.65%	Teflubenzuron	3.83%	Myclobutanil	4.7%	BaA	8.51%
Pyrimethanil	3.65%	Endosulfan Sulfate	3.69%	Tebuconazole	4.62%	DbaeP	5.73%
Secoisolariciresinol	3.29%	Bifenthrin	3.62%	Thiophanate methyl	4.41%	Niv	1.02%
Daidzeine	2.59%	Pyriproxyfen	3.46%	Triadimenol	4.26%	PCDD 12378	0.83%
Coumestrol	2.5%	Diethofencarb	3.24%	Dimethoate	4.25%	FA	0.42%
Genisteine	2.3%	Captan	2.97%	Phosalone	4.24%	Sr	0.4%
Cyproconazole	1.77%	DbaIP	2.91%	Endosulfan Beta	4.16%	Cu	0.38%
Glyciteine	1.03%	Chlorothalonil	2.86%	PAT	4,00%	Genisteine	0.23%
Ca	1.02%	15 Ac DON	2.73%	Azinphos methyl	3.99%	Resveratrol	0.23%
Chlorothalonil	1.01%	Chlortal dimethyl	2.7%	Mepanipyrim	3.87%	Ga	0.21%
Li	0.94%	Chlorfenvinphos	2.55%	Imazalil	3.29%	Ethion	0.19%
Sn	0.6%	Carbofuran	2.38%	Triflumuron	2.6%	PFOA	0.17%
DbaiP	0.47%	DbaiP	2.36%	Folpet	2.6%	PFHpA	0.16%
N = 25 Sp = 0.87 %Ve = 3.48		N = 53 Sp = 0.73 %Ve = 7.96		N = 27 Sp = 0.86 %Ve = 5.28		N = 25 Sp = 0.87 %Ve = 1.12	

%contrib = statistical weight of each substance in the mixture (matrix U) divided by the sum of the weights of all substances in the mixture multiplied by 100;
N = number of substances with a statistical weight > 0 in the mixture;
Sp = sparsity of the mixture;
%Ve = percentage of variance explained by the mixture;
The horizontal line limits the substances that contributes the most to the mixture (up to a sum of 50% and/or that alone contribute to at least 5%).

each mixture. The correlation matrix between the weight of adherence to mixtures and the consumption of the main food groups is presented in Table 2.

The average exposure to the substances that contribute the most to each mixture as well as the mean daily consumption (g/day) of the 24 main food groups according to the quartile groups of adherence to the 8 mixtures are given in **Tables S1-S8**. These tables also present the distribution of the quartiles of the mixtures by clusters.

Each mixture will now be described mainly focusing on the substances that contribute the most to the mixture (up to a sum of 50%) and/or that alone contribute to at least 5% of the mixture.

- Mixture 1: Minerals, inorganic contaminants, and furans

The first mixture was mainly composed by minerals (Cr, K, Fe, Zn, Mo), inorganic contaminants (Pb, Cd, Ba, Ni, V) and furans (HCDF_1234789, 1234678, 123478, 234678, 123678, OCDF, PCDF_23478). For mixture 1 high correlation (rho > 0.15) was highlighted with the consumption of several food groups. The three food groups for which the highest correlation was reported were "Offal" (rho = 0.22), "Vegetables except roots" (rho = 0.20), and "Eggs" (rho = 0.19).

- Mixture 2: PCB, furan, and BFR

PCB-DL (PCB-118, 189, 157, 114, 167, 105, 169) and PCB_NDL (PCB-138, 153) mainly characterized the second mixtures, although also PCDF-12378 (a furan), and HBCD-alpha, beta and gamma (BFR), contributed to this mixture. "Butter and cream" (rho = 0.20), and "Cheese" (rho = 0.17) consumption had the high correlation with this mixture.

- Mixture 3: Mycotoxins, pesticides, and PAH

Mycotoxins (deoxynivalenol, trichothécènes des groupes A (toxine HT-2) and B (nivalenol, deoxynivalenol), ocratoxin A, zéaralénone), two pesticides, namely Pirimiphos-methyl and Piperonyl-butoxide, and four PAH (namely Bghip, CPP, BjF, BbF) were the main components of mixture 3. This mixture presented a very high correlation with consumption of "Bread and salty cereal products" (rho = 0.73), and in lower extent, with consumption of "Starch food" (rho = 0.25), and "Cakes and sweet cereal products" (rho = 0.17).

- Mixture 4: PCB, BFR, furans, Hg, and PFAS

The fourth mixture was composed by PCB_101 (a PCB-DL), PBDE154, 28, 100, PBB153 and 52 (BFR), TCDF_2378 (a furan), Hg, as well as PFOS, PFUnA and PFTrDA (PFAS). A very high correlation was highlighted between mixture 4 and consumption of "Fish" (rho = 0.64), and, to a lower extent, consumption of "Offal" (rho = 0.18), "Fat except butter and cream" (rho = 0.17), and "Vegetables except roots" (rho = 0.17).

- Mixture 5: Pesticides (1)

The fifth mixture was characterized mainly by pesticides such as, Iprodione, Lambda Cyhalothrin, Fludioxonyl, Sulfur, Cyprodinyl, Procymidone, and Boscalid. Consumption of "Vegetables except roots" (rho = 0.37) and "Roots" (rho = 0.25) were the food groups with a high correlation to mixture 5.

- Mixture 6: Pesticides (2)

Table 2
Partial Spearman correlations between daily consuption of food groups and weights of adherence to mixtures in E3N cohort (n = 73 031).

Food groups daily consumption (g/day)	Adherence to mixtures*							
	Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5	Mixture 6	Mixture 7	Mixture 8
	Minerals, inorganic contaminants, and furans	PCB, furan, and BFR	Mycotoxins, pesticides, and PAH	PCB, BFR, furans, Hg, and PFAS	Pesticides (1)	Pesticides (2)	Pesticides (3)	Mycotoxins and PAH
Bread and salty cereal products	**−0.14**	**0.04**	**0.73**	0.01	**0.1**	0.00	**0.16**	**0.13**
Butter and cream	**0.05**	**0.2**	**0.13**	**−0.22**	**−0.08**	**0.05**	**−0.12**	**0.03**
Cakes and sweet cereal products	**0.12**	**−0.08**	**0.17**	**0.07**	**−0.04**	**0.08**	**0.01**	**0.03**
Cheese	**0.11**	**0.17**	**−0.02**	**−0.24**	**−0.04**	0.00	**−0.04**	**−0.02**
Coffee	**0.07**	**−0.02**	**−0.04**	0.00	**0.02**	**0.09**	**−0.05**	0.00
Eggs	**0.19**	**−0.05**	**−0.08**	**0.05**	**−0.01**	**0.04**	**−0.07**	**0.01**
Fish	**−0.04**	**−0.08**	**−0.02**	**0.64**	**0.08**	**0.02**	**0.15**	**0.00**
Alcohol	**0.09**	**−0.04**	0.01	**0.06**	0.00	**0.09**	**−0.07**	**0.02**
Fresh dairy	**0.16**	**0,00**	**−0.23**	**−0.04**	**−0.04**	0.00	**−0.03**	**−0.01**
Fruits	**0.12**	**−0.1**	**−0.1**	**0.1**	**0.06**	**0.01**	**0.72**	**0.02**
Offal	**0.22**	**−0.16**	**−0.12**	**0.18**	**−0.04**	**0.07**	**−0.01**	**−0.02**
Fat except butter and cream	**0.16**	**−0.11**	**−0.01**	**0.17**	**0.08**	**0.05**	**0.01**	**0.04**
Charcuterie	**0.14**	**−0.01**	**0.05**	**−0.01**	**−0.08**	**0.11**	**−0.08**	**−0.02**
Red meat	**0.06**	**0.1**	**−0.05**	**−0.13**	**−0.05**	**0.06**	**−0.07**	**−0.06**
White meat	**0.09**	**0.1**	**−0.03**	**−0.12**	**−0.03**	**0.04**	**−0.08**	**−0.04**
Seafood	**−0.02**	**0.04**	**0.07**	**0.14**	**0.02**	**0.1**	**0.04**	0.00
Soda	**0.05**	**−0.02**	**0.03**	**0.01**	**−0.04**	**0.05**	0.00	**−0.02**
Starch food	**0.12**	**−0.06**	**0.25**	**0.07**	**−0.02**	**0.07**	0.00	**0.05**
Sugar products	**0.17**	**−0.12**	**0.14**	**0.06**	**−0.05**	**0.05**	**0.05**	**0.03**
Sweet diary	**0.12**	**−0.03**	**0.06**	**0.01**	**−0.09**	**0.07**	**−0.09**	**0.01**
Teas	**0.02**	**−0.02**	**0.02**	**0.06**	**0.02**	0.00	**0.04**	**0.03**
Roots	**0.13**	**−0.1**	**−0.13**	**0.13**	**0.25**	**0.12**	**0.15**	0.00
Vegetables except roots	**0.2**	**−0.17**	**−0.16**	**0.17**	**0.37**	**0.05**	**0.14**	**0.03**
Water	**0.07**	**−0.06**	**−0.05**	**0.09**	**0.07**	**0.02**	**0.04**	**−0.02**

Partial Spearman rank correlation coefficients between each mixtures and each food groups, adjusted on other mixtures.
In **bold**: Significantly non-zero Spearman partial rank correlation coefficient (p value < 0.05).
* Statistical weight of adherence of individuals to mixtures (matrix V).

Mixture 6 was mainly composed by pesticides, although different from those identified in the fifth mixture: Bupirimate, Phosmet, Carbendazim, Kresoxim methyl, Vinclozolin, Chlorpyrifos methyl, Quinoxyfen, Teflubenzuron, Endosulfan Sulfate, Bifenthrin, Pyriproxyfen, and Diethofencarb. This mixture wasn't strongly correlated with the consumption of any food group. The highest correlation was found with the food groups "Roots" (rho = 0.12), followed by "Charcuterie" (rho = 0.11) and "Seafood" (rho = 0.10).

- Mixture 7: Pesticides (3)

Fenhexamid, Thiabendazole, Azoxystrobin, Diphenylamine, Propargite, Chlorpyrifos ethyl, Acrinathrin, Myclobutanil, Tebuconazole, and Thiophanate methyl, were the pesticides that contributed most to the seventh mixture. This mixture presented a very high correlation with consumption of "Fruit" (rho = 0.72), and less markedly with "Bread and salty cereal products" (rho = 0,17).

- Mixture 8: Mycotoxins and PAH

The last mixture, similarly to mixture 3, was composed mainly by mycotoxins (ochratoxin B, alpha-zearalanol and alpha-zearalenol, diacetoxyscirpenol, fusarenon X, 3-acetyldeoxynivalenol, trichothecenes from group A, such as T2-toxin) and PAH (BaA and DbaeP). This mixture wasn't strongly correlated with the consumption of any food group. The highest correlation was found with the food group "Bread and salty cereal products" (rho = 0.13).

3.2. Description of clusters in the E3N cohort based to the adherence to the identified chemical mixtures

Finally, 5 clusters, identified based on the adherence to the 8 mixtures, were retained on the basis of the relevance of the clusters obtained. For each cluster, the average adherence and contribution of each mixture are described in Fig. 1 and in Table 3 together with the main characteristics of the cluster population in comparison to the overall study population.

The first cluster included 7% (5136 women) of the study population. The average age and BMI of women included in this cluster were 53.0 years and 22.1 kg/m^2, respectively. Women included in the first cluster were slightly more active (47.3 metabolic equivalent of task (MET). hours/week) and less frequently ever smokers (42.2%) compared to the overall study population, and 34.9% had more than 14 years of school education. Cluster 1 was characterized by a remarkably higher adherence to the mixture "Mycotoxins and PAH". The mixtures that contributed the most to cluster 1 were: "Minerals, inorganic contaminants, and furans" (21.0%), "Mycotoxins and PAH" (17.4%), and "Mycotoxins, pesticides, and PAH" (14,4%).

The second cluster included 27 685 women representing 38% of the study population. Women included in this cluster had an average age of 53.6 years, and, in comparison with the overall population, had a higher BMI (23.9 kg/m^2) and were less physically active (43.6 MET.hours/week). Among cluster 2, 45.0% women were ever smokers and 33.7% had more than 14 years of school education. Women in cluster 2 had a lower average adherence to all mixtures compared to the overall study population, with the exception of mixture "Pesticides (2)". Looking at the relative contribution of the mixtures to the cluster, the mixtures "Minerals, inorganic contaminants & furans" (25.3%), "Pesticides (2)" (17.6%), and "PCB, furan and BFR" (16.2%) were the mixtures that contributed the most.

Cluster 3 included 16 622 women (23%) which were on average younger (51.4 years old), slimmer (BMI 22.1 kg/m^2), less physically active (44,5 MET.hours/week), and more frequently ever smokers (47,5%), compared to the overall study population. In cluster 3, a higher proportion of women had more than 14 years of school education (39.3%). Cluster 3 was characterized by a higher adherence to the mixtures "Minerals, inorganic contaminants & furans", "PCB, furan, and BFR " and "Mycotoxins, pesticides, and PAH" compared to the overall study population. These mixtures were also those that had the highest relative contribution to cluster 3: "Minerals, inorganic contaminants & furans" (25.8%), "PCB, furan, and BFR" (16.9%), and "Mycotoxins, pesticides, and PAH" (19.5%).

Women included in the fourth cluster represented 10% (n = 7424) of the study population. Women in cluster 4, 52.9 years old on average with a BMI of 22.4 kg/m^2, were more physically active (52.4MET.hours/week) compared to the overall population, more frequently ever

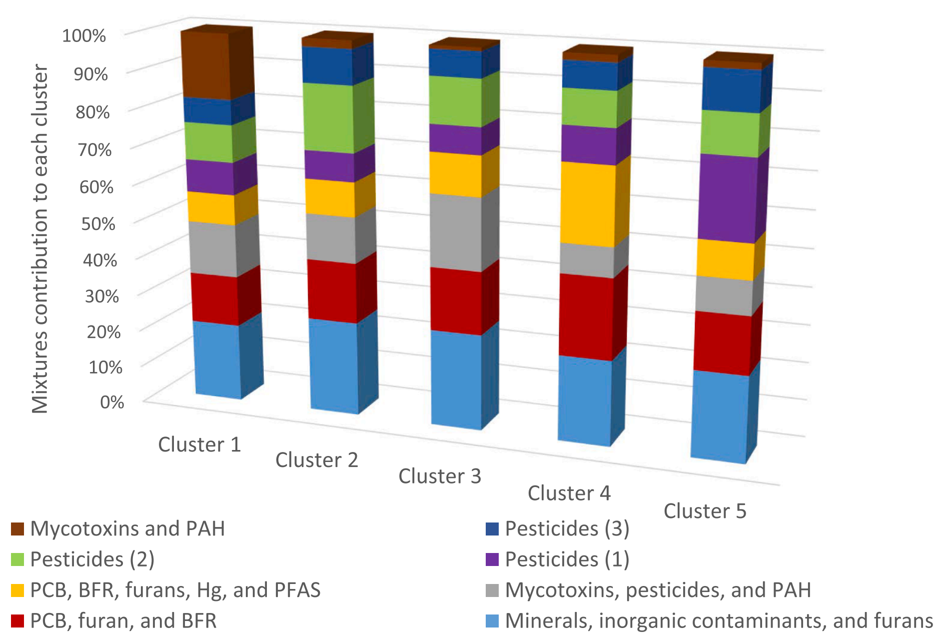

Fig. 1. Mixtures contribution (in percentage) to each cluster identified in the E3N cohort (n = 73 031). For each cluster, the contributions of each mixture (in percentages) are calculated as follows: contribution of mixture 1 to cluster 1 = (average weight of adherence to mixture 1 in cluster 1 / (average weight of adherence to mixture1 in cluster 1 + average weight of adherence to mixture 2 in cluster 1 + [...] + average weight of adherence to mixture 8 in cluster 1)) * 100.

Table 3
Description of the cluster identified in E3N cohhort (main characteristics, adherence to mixtures and mixtures contribution).

Clusters	Description		P value (1)	Average adherance to the mixture (2)		P value (3)	Mixtures contribution (4)
Overall study population	N	73,031 (100%)					
	Age	52.9 years		Minerals, inorganic contaminants, and furans	3.66		24.14
	BMI	22.9 kg/m^2		PCB, furan, and BFR	2.53		16.69
	Physical activity	46.4 MET.hours/week		Mycotoxins, pesticides, and PAH	1.97		12.99
	Ever smoking	46.0%		PCB, PFAS, BFR, Hg, furans	1.68		11.08
	Education level			Pesticides (1)	1.66		10.95
	<BAC	11.3%		Pesticides (2)	1.93		12.73
	BAC to BAC + 2	52.9%		Pesticides (3)	1.26		8.31
	≥BAC + 3	35.9%		Mycotoxins and PAH	0.47		3.10
1	N	5136 (7%)					
	Age	53.0 years	0.49	Minerals, inorganic contaminants, and furans	4.09	<0.0001	21.01
	BMI	22.1 kg/m^2	<0.0001	PCB, furan, and BFR	2.64	<0.0001	13.56
	Physical activity	47.3 MET.hours/week	0.1794	Mycotoxins, pesticides, and PAH	2.8	<0.0001	14.38
	Ever smoking	42.2%	<0.0001	PCB, BFR, furans, Hg, and PFAS	1.59	<0.0001	8.17
	Education level		0.3392	Pesticides (1)	1.71	0.0028	8.78
	<BAC	11.3%		Pesticides (2)	1.95	0.0763	10.02
	BAC to BAC + 2	53.8%		Pesticides (3)	1.31	<0.0001	6.73
	≥BAC + 3	34.9%		Mycotoxins and PAH	3.38	<0.0001	17.36
2	N	27,685 (38%)					
	Age	53.6 years	<0.0001	Minerals, inorganic contaminants, and furans	2.87	<0.0001	25.33
	BMI	23.9 kg/m^2	<0.0001	PCB, furan, and BFR	1.84	<0.0001	16.24
	Physical activity	43.6 MET.hours/week	<0.0001	Mycotoxins, pesticides, and PAH	1.41	<0.0001	12.44
	Ever smoking	45.0%	0.0005	PCB, BFR, furans, Hg, and PFAS	1.05	<0.0001	9.27
	Education level		<0.0001	Pesticides (1)	0.86	<0.0001	7.59
	<BAC	12.8%		Pesticides (2)	1.99	<0.0001	17.56
	BAC to BAC + 2	53.5%		Pesticides (3)	1.06	<0.0001	9.36
	≥BAC + 3	33.7%		Mycotoxins and PAH	0.25	<0.0001	2.21
3	N	16,622 (23%)					
	Age	51.4 years	<0.0001	Minerals, inorganic contaminants, and furans	4.39	<0.0001	25.01
	BMI	22.1 kg/m^2	<0.0001	PCB, furan, and BFR	2.87	<0.0001	16.87
	Physical activity	44.5 MET.hours/week	<0.0001	Mycotoxins, pesticides, and PAH	3.31	<0.0001	19.46
	Ever smoking	47.5%	<0.0001	PCB, BFR, furans, Hg, and PFAS	1.83	<0.0001	10.76
	Education level		<0.0001	Pesticides (1)	1.22	<0.0001	7.17
	<BAC	9.2%		Pesticides (2)	2.07	<0.0001	12.17
	BAC to BAC + 2	51.5%		Pesticides (3)	1.16	<0.0001	6.82
	≥BAC + 3	39.3%		Mycotoxins and PAH	0.16	<0.0001	0.94
4	N	7424 (10%)					
	Age	52.9 years	0.8888	Minerals, inorganic contaminants, and furans	4.7	<0.0001	22.85
	BMI	22.4 kg/m^2	<0.0001	PCB, furan, and BFR	4.43	<0.0001	21.54
	Physical activity	52.4 MET.hours/week	<0.0001	Mycotoxins, pesticides, and PAH	1.63	<0.0001	7.92
	Ever smoking	50.3%	<0.0001	PCB, BFR, furans, Hg, and PFAS	4.24	<0.0001	20.61
	Education level		<0.0001	Pesticides (1)	1.9	<0.0001	9.24
	<BAC	9.6%		Pesticides (2)	1.88	<0.0001	9.14
	BAC to BAC + 2	49.6%		Pesticides (3)	1.41	<0.0001	6.85
	≥BAC + 3	40.8%		Mycotoxins and PAH	0.38	<0.0001	1.85
5	N	16,164 (22%)					
	Age	53.3 years	<0.0001	Minerals, inorganic contaminants, and furans	3.66	0.7985	22.94
	BMI	22.6 kg/m^2	<0.0001	PCB, furan, and BFR	2.43	<0.0001	15.23
	Physical activity	50.0 MET.hours/week	<0.0001	Mycotoxins, pesticides, and PAH	1.43	<0.0001	8.96
	Ever smoking	45.5%	0.1744	PCB, BFR, furans, Hg, and PFAS	1.47	<0.0001	9.21
	Education level		<0.0001	Pesticides (1)	3.36	<0.0001	21.06
	<BAC	11.7%		Pesticides (2)	1.69	<0.0001	10.59
	BAC to BAC + 2	54.3%		Pesticides (3)	1.64	<0.0001	10.28
	≥BAC + 3	34.1%		Mycotoxins and PAH	0.27	<0.0001	1.69

(1) p value of *t*-test (for quantitative variables) or chisq test (for categorical variables) comparing the mean (for quantitative variables) or proportion (for categorical variables) of the variable between the cluster and the general population.
(2) Statistical weight of adherence of individuals to mixtures (matrix V).
(3) p value of *t*-test comparing the mean weight of adherence to each mixture between the cluster and the general population.

(4) for each cluster, the contributions of each mixture (in percentage) are calculated as follows: contribution of mixture 1 to cluster 1 = (average weight of adherence to mixture 1 in cluster 1 / (average weight of adherence to mixture1 in cluster 1 + average weight of adherence to mixture 2 in cluster 1 + […] + average weight of adherence to mixture 8 in cluster 1)) * 100.

smokers (50.3%), and with a higher average education level (40.8% had more the 14 years of school education). A higher adherence to mixtures “Minerals, inorganic contaminants, and furans”, “PCB, furan, and BFR”, and “PCB, BFR, furans, Hg, and PFAS” characterized cluster 4 in comparison to the study population. Concerning the relative contribution to cluster 4, the mixtures “Minerals, inorganic contaminants, and furans” (22.9%), “PCB, furan, and BFR” (21.5%), and “PCB, PFAS, BFR, Hg, furans” (20.6%) were also those that contributed the most.

Finally, cluster 5 included 16 164 (22%) women, 53.3 years old on average, with a BMI of 22.6 kg/m^2 and a physical activity of 50.0 MET. hours/week. Among women included in cluster 5, 45.5% were ever smokers and 34.1% had over 14 years of education. Women in the fifth cluster had a higher adherence to the mixture “Pesticides (1)” and a lower adherence to the mixture “Mycotoxins, pesticides, and PAH” when compared to the overall study population. “Minerals, inorganic contaminants & furans” (23.0%) followed by “Pesticides (1)” (21.1%), and “PCB, furan, and BFR” (15.2%) were the mixtures that had the highest relative contribution to this cluster.

4. Discussion

This study made it possible to summarize the total exposure of the E3N cohort by 8 main chemical mixtures to which these women are exposed through their diet and to quantify the average dietary exposure to the substances that contributed the most to each mixture. Moreover we also identified and described 5 cluster groups of women with similar profiles of co-exposure to these 8 chemical mixtures.

Organic and inorganic minerals are the main components of the first mixture together with furans. The first mixture is correlated with the consumption of offal, such as liver, which represents a storage compartment and thus an important source of dietary exposure especially to organic and inorganic minerals (Abd-Elghany et al., 2020).

The second mixtures is greatly characterized by exposure to PCB, and is mainly correlated with consumption of dairy products which have already been identified as the main dietary sources of PCB for the French population (Sirot et al., 2012). PCBs are persistent and bioaccumulative lipophilic substances, mostly used in the past by industry. PCBs have been associated with numerous adverse health effects and were classified as probably carcinogenic to humans by the International Agency for Research on Cancer (IARC).

Consumption of cereal based and starchy products is highly correlated with exposure to the third mixture which is mainly composed by mycotoxins and PAH. Exposure to mycotoxins and PAH characterize also mixture 8 which is as well correlated to consumption of cereal products, although to a lower extent compared to mixture 3. Mycotoxins are secondary metabolites produced by the toxinogenic strains of several fungi species and enter the food chain as a result of infection of crops before or after harvest and are typically found in foods such as cereals and spices (Marin et al., 2013). PAH are a large group of organic compounds suspected to be cancerogenic and genotoxic, significantly present in food, such as bread and cereal products, due to heat processes such as grilling and baking (Veyrand et al., 2013).

PFAS, BFR, and furans, as well as Hg characterize mixtures 3. This mixture is highly correlated with consumption of fish. Fish is a healthy food choice, however, some predatory fish accumulate particularly high levels of toxic substances, such as persistent organic pollutants (e.g. PFAS, BFR, and furans) and Hg, which may potentially induce various health problems such as endocrine disruption, cardiovascular diseases, cancers, diabetes, birth defects, and dysfunctional immune and reproductive systems (Guo et al., 2019; Woolorton, 2002).

Exposure to pesticides characterize mixtures 5, 6, and 7, and, as expected, these mixtures are correlated mainly with consumption of vegetables and roots (mixture 5 and 6) and fruit (mixtures 7) (Nougadère et al., 2012).

When looking at the results of the cluster analysis, although it shows how the first mixture (“Minerals, inorganic contaminants, and furans”) represents the major contributor to each clusters, it also highlights different patterns of exposure per cluster, despite the quite homogeneous study population based on middle aged French women with a fairly high level of education.

The results obtained by the present study have a double usefulness. Firstly, these results can help prioritize which substances should be considered together as mixtures when performing risk assessment. It also has to be considered that combined effects of chemicals can occur in a mixture even when they are present at concentrations where the individual chemicals show no effect; thus it is crucial to test combined chemicals at doses to which the population is realistically exposed (Ten, 2007). Providing levels of dietary exposure to chemicals which compose the identified mixtures, the present study may represent a reference for future toxicology studies.

Secondly, this work represents the basis to perform epidemiological studies to investigate the relationship between long-term dietary exposure to chemical mixtures and health outcomes in the E3N cohort. Understanding the health effects of exposure to real-world concentrations of chemical mixtures is challenging not only due to the difficulties encountered in assessing exposure, but also due to the fact that humans may experience exposure to non-chemical stressors simultaneously and because genetics may affect susceptibility of population subgroups (Hernández and Tsatsakis, 2017). With nearly 100 000 participants and over 25 years of detailed follow-up, the E3N cohort represents a privileged research setting for studying the long terms effect of dietary exposure to chemical mixtures in adult women. Ultimately epidemiological observations may generate hypotheses of associations between exposure and health outcome which may direct experimental studies to highlight the underlying biological mechanisms.

Other studies have attempted to identify the major chemical mixture to which the population is exposed through the diet. A similar approach to the one followed in the present study has previously been adopted in EDEN and ELFE cohorts to identify the main chemical mixtures to which pregnant women are exposed through the diet in France (Traoré et al., 2018). There is some degree of similarity among the chemical mixtures identified in the three cohorts. In particular, as the mixture 1 in E3N cohort, a mixture composed mostly of trace elements and furans, was also identified from EDEN and ELFE cohorts. A mixture characterized by exposure mainly to PCBs was identified in the three cohorts, as well as three mixtures composed by pesticides, although with different groupings. The differences in the results obtained for E3N cohort compared to those obtained for the cohorts EDEN and ELFE are most probably due to the differences in terms of dietary habits between the study populations. While E3N cohort is composed of middle-aged women (53 years old on average in 1993), EDEN and ELFE cohorts include pregnant women: indeed pregnancy represents a particular period of life during which dietary habits are frequently modified in order to meet the dietary recommendation specific for pregnant women.

Also Traoré et al. (2016) investigated the main chemical mixtures to which the French population is exposed through the diet, based on a sample of 2624 adults aged 18–79 years, selected to be representative of the entire French population (Traoré et al., 2016). Due to some methodological differences between the two studies, it is not possible to directly compare their results, nevertheless both studies show how consumption of fruit and vegetables is associated with higher exposure to pesticides.

4.1. Strengths and limitations

Some limitations have to be taken into account when interpreting the results of the present study. The E3N cohort, like most cohort studies, is not representative of the French middle-aged women population. Therefore extrapolating results to the general population must be done carefully. The approach used in this study only focuses on chemicals chosen to be considered in the analyses or present at concentration above the limit of quantification. As a consequence, only a small part of the overall chemicals is addressed, and chemicals that were not included in the analysis cannot be identified as part of the mixtures. Moreover, food contamination levels may change over time and this variability was not taken into account in the study since the contamination data published by Anses in the TDS2 represent the contamination levels at a given time (2007–2009). The time gap between the E3N food questionnaire (1993) and the food sample collection carried out for TDS2 has to be also taken into account as a potential limitation since food contamination level and/or food consumption habits could have varied over time. In the framework of the present studies where dietary exposure to multiple substances is considered simultaneously, it is not straightforward to predict the overall impact of the variation in food contamination levels on the results since contamination levels of certain substances may have increased while other may have decreased. Concerning potential changes in food consumption habits, previous evidence suggest that only minor changes in middle-aged women's dietary patterns occur over time, although the overall amount of food intake tends to decrease (Thorpe et al., 2019). Thus we can assume the impact of dietary changes to be limited on the results of the present study. Another potential source of uncertainty is related to the matching between the food consumption data of the E3N cohort and the food contamination data of the TDS2 which use different food nomenclatures and thus may represent a source of uncertainty. Nevertheless, both the E3N dietary questionnaire and the list of food items included in the TDS2 were selected based on the French dietary pattern, so that finally only small differences were found with realistically a limited impact on the estimates. Due to the fact that dietary consumption data are self-reported, a certain degree of misclassification of exposure is possible. Finally, when interpreting the results it has to be taken into account that the cluster analysis is performed directly from the V matrix obtained by the SNMU, thus ignoring the error produced by factorization.

This study also presents several strengths. With more than 70 000 women included, this study is the largest ever conducted to identify dietary exposure to chemical mixtures. Despite the limitation listed above, consumption data have been collected with previously validated food questionnaires which strengthens our confidence in the results. Moreover, data provided by the TDS2 are the most complete and up to date contamination data in food as consumed by the French population, allowing to study the exposure to more than a hundred substances at the same time. Finally, the method used in the present study is an innovative approach: indeed the Principal Component Analysis traditionally used to reduce dimension is not totally adapted for exposure data, as latent variables and noise are modeled with a normal distribution in this method. SNMU is a method of reduction of dimensions specifically developed for non-negative matrix of real numbers, and thus adapted to positive data with excess zeros values, such as dietary exposure data (Bechaux et al., 2013). Moreover, contrary to the previously used non-negative matrix factorization (NMF), the SNMU presents the advantage of providing a unique optimal solution, thus stabilizing the results and facilitating their interpretation (Gillis and Glineur, 2009; Gillis and Plemmons, 2013).

4.2. Conclusion

This study highlighted the chemical mixtures to which middle-aged French women are realistically exposed through the diet, representing a concrete attempt to prioritize mixtures for which it is essential to investigate combined health effects based on exposure. Moreover the present study provides a methodological framework to further investigate the epidemiological link between the exposure to the identified mixtures and long term health effects generating hypothesis which may direct future toxicological studies.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106467.

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