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To which chemical mixtures is the French population exposed? Mixture identification from the second French Total Diet Study

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ABSTRACT

Through their diet, humans are exposed to a wide range of substances with possible adverse effects. Total diet studies (TDS) assess exposure and risk for many single substances or mixtures from the same chemical family.

This research aims to identify from 440 substances in the second French TDS, the major mixtures to which the French population is exposed and their associated diet. Firstly, substances with a contamination value over the detection limit were selected. Secondly, consumption systems comprising major consumed foods were identified using non-negative matrix factorisation and combined with concentration levels to form the main mixture. Thirdly, individuals were clustered to identify "diet clusters" with similar consumption patterns and co-exposure profiles.

Six main consumption systems and their associated mixtures were identified. For example, a mixture of ten pesticides, six trace elements and bisphenol A was identified. Exposure to this mixture is related to fruit and vegetables consumed by a diet cluster comprising 62% of women with a mean age of 51 years. Six other clusters are described with their associated diets and mixtures. Cluster co-exposures were compared to the whole population.

This work helps prioritise mixtures for which it is crucial to investigate possible toxicological effects.

1. Introduction

Due to the large number of chemicals found in the environment, individuals are daily exposed to complex mixtures of chemicals which can interact and cause health problems. One of the main/major challenges facing risk assessment is the risk related to mixtures, a particularly difficult task due to the multitude of possible combinations of chemicals for which it is unrealistic to test combined toxicological effects. For this reason, risk is usually assessed for chemicals belonging to the same chemical family (such as dioxins and furans (Van den Berg et al., 2006) or triazoles (EFSA, 2009b)), that share a similar mode of action (Bosgra et al., 2009; Kortenkamp and Faust, 2010). It may also be assessed by grouping substances by their organ toxicity or specific effects (EFSA, 2013; RIVM et al., 2016). Recently, Orton et al. (2014) proposed an approach based on a combination of 30 androgen receptor antagonists composed of 13 pesticides and 17 non pesticides. The mixture analysed in Orton's study contained substances with similar mechanisms of action and covered a wide range of sources and exposure routes, but the co-occurrence of their exposure was not considered. Thus, one important question remains: do the mixtures defined on the basis of toxicological properties reflect the reality of exposure? The present work proposes to identify chemical mixtures from individuals' exposure to different foods.

Dietary exposure is commonly assessed by combining data on the quantity of food consumed with chemical concentrations in the food. Total diet studies (TDS) aim to provide data on concentrations in food consumed by the general population for a wide range of substances as well as the corresponding exposure. The second French TDS (hereafter referred to simply as TDS 2) (Sirot et al., 2009) investigated around 440 chemicals and used consumption data provided by INCA 2, the second French national food consumption survey (Dubuisson et al., 2010; Lioret et al., 2010). One hundred and fifty-three substances were detected in at least one food, showing the multitude and diversity of substances to which the French population is exposed through its diet. As it is not realistic to test all the possible combinations of these substances for

Abbreviations: BMI, body mass index; CS, consumption system; HCPC, Hierarchical Clustering on Principle Components; INCA, Individual and National Food Consumption Survey; LB, lower bound; LOD, limit of detection; LOQ, limit of quantification; NMF, non-negative matrix factorisation; TDS, Total Diet Study.

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their combined effects, it is important to extract the main mixtures to be evaluated as a priority by toxicological and epidemiological studies.

Crépet and co-authors (Crépet and Tressou, 2011; Crépet et al., 2013a, 2013b) proposed a statistical method based on a Bayesian non-parametric model to determine major mixtures from dietary exposures. This method is used to classify the population regarding their exposure profiles and then study correlations between pesticide exposures to define mixtures. A second approach presented in Béchaux et al. (2013) and based on non-negative matrix factorisation (NMF) (Lee and Seung, 2001), consists in reducing the size of the dataset before classification. This work was conducted on 26 priority pesticides from TDS 2. The peculiarity of this approach was to characterise not only the mixtures but also the main foods contributing to exposure to these mixtures. Indeed, the NMF method has previously been used to define dietary patterns and clusters of individual diets by Zetlaoui et al. (2011), Sy et al. (2013) and, more recently, Gazan et al. (2016).

The objective of this work was to apply the approach based on NMF (Béchaux et al., 2013) to the 153 substances detected in TDS 2 (some of which contained pesticides and others not) in order to identify chemical mixtures. The NMF analysis was completed by hierarchical clustering to classify groups with similar dietary behaviour and a similar co-exposure profile. The defined groups were characterised by socio-demographic data (age, body mass index and the monthly household income) and levels of exposure to the substances in the mixtures. Thus, this analysis of the 153 substances led to the definition of the major chemical mixtures to which the French population is exposed and their main food vectors.

2. Materials and method

2.1. Consumption data from the "Individual and National Food Consumption Survey", INCA2

The individual food consumptions were provided by the second "Individual and National Food Consumption Survey", i.e. the INCA 2 survey carried out by the French Food Safety Agency between late 2005 and April 2007. Two independent random samples were included in this survey: 1,455 children aged 3 to 17 years (Lioret et al., 2010) and 2,624 adults aged 18 to 79 years (Dubuisson et al., 2010). Participants were selected to be representative of the French population using a three-stage random probability design stratified by region of residence, size of urban area and population category (adults and children) then a sampling weight was attributed to each individual. Subjects completed a seven-day food record diary and portion sizes were estimated through photographs compiled in a manual adapted from the Su-Vi-Max photographic booklet (Hercberg et al., 1994) or expressed by weight or household measures. Thus, the quantities of 1,280 food items consumed per day were recorded. Demographic and socio-economic variables were collected for each individual: age, body mass index (BMI) and the household monthly income. The latter was divided into three categories: "low": less than 1,300 euros; "medium": between 1,300 and 3,100 euros; and "high": more than 3,100 euros. Only adults were considered in this study, and subjects with an extremely low total energy intake were excluded. *In fine*, the research design resulted in a sample of 2,607 adults.

2.2. Concentration data from the second French Total Diet Study

TDS 2 (Sirot et al., 2009) provides the concentration of 440 substances in 212 core foods (Arnich et al., 2012; Bemrah et al., 2012; Nougadère et al., 2012; Sirot et al., 2012a, 2012b, 2013; Veyrand et al., 2013; Rivière et al., 2014). The latter, defined from the classification of the 1,280 INCA 2 food items, cover about 90% of the whole diet. Food samples were collected in eight French regions (36 cities) and prepared 'as consumed' to be analysed for their mineral composition and contamination levels. In order to be representative of French population habits, each sample was composed of 15 sub-samples of the same food, so as to cover different varieties, purchase locations, preparation methods, cooking etc. In all, 19,785 different food products were purchased over different seasons from 2007 to about 2009 to make up the 1,319 composite samples of core foods to be analysed for additives, environmental contaminants, pesticide residues, trace elements and minerals, mycotoxins, phytoestrogens and acrylamide. In the present work, the core foods consumed by less than 5% of the population were excluded to avoid emphasising a dietary behaviour that was too specific or isolated. Thus, 177 core foods were considered in the analysis.

Concentrations below the analytical limits of detection (LOD) or quantification (LOQ) could not be detected and/or quantified. These data are said to be left-censored, and one solution is to replace censored data by a fixed value (GEMS-Food Euro, 1995) according to different scenarios. In order to focus on substances with a detected value, the LB (Lower Bound) scenario was considered. This consists in replacing non-detected values by 0, and detected values unable to be quantified, by the LOD. With this scenario, the exposure of the whole population is equal to zero for 210 pesticides, four perfluorinated compounds and seven mycotoxins that were not considered in this study. The 12 minerals analysed in TDS 2 were not considered either so as to focus on contaminants with adverse effects. The remaining 207 out of 440 substances were considered single or were summed by congeners:

- ➢ 18 trace elements: aluminium (Al), antimony (Sb), barium (Ba), cadmium (Cd), cobalt (Co), gallium (Ga), germanium (Ge), lead (Pb), nickel (Ni), silver (Ag), strontium (Sr), tellurium (Te), tin (Sn) and vanadium (V). Separate analyses were performed to take into account the proportion of inorganic and organic arsenic (As) and mercury (Hg). Inorganic and organic arsenic (Asi and Aso) and inorganic and organic mercury (Hgi and MeHg) are therefore considered instead of total arsenic and mercury;
- ➢ the sum of 17 congeners of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCCD/F) (HCDD/F-123478, HCDD/F-123678, HCDD/F-123789, HCDD/F-1234678, OCDD/F, PCDD/F-12378, TCDD/F-2378, HCDF-1234789, HCDF-234678, PCDF-23478) and 12 congeners of 'dioxin-like' polychlorinated biphenyls (DL-PCBs) (PCB-77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189): *DIOX_PCB*;
- ➢ the sum of six congeners of 'non-dioxin-like' polychlorinated biphenyls (NDL-PCBs) (PCB-28, 52, 101, 138, 153, 180): *PCBi6*;
- ➢ 12 perfluorinated compounds: nine carboxylates (PFDA, PFDoA, PFHpA, PFHxA, PFNA, PFOA, PFTrDA, PFTrDA and PFUnA) and three sulphonates (PFBS, PFHxS and PFOS);
- ➢ three sums of brominated flame retardants (BFRs): *sum8PBDE* of eight polybrominated diphenyl ether congeners (PBDE-28, 47, 99, 100, 153, 154, 183, and 209), *sumPBB* of three polybrominated biphenyl congeners (PBB-52, 11 and 153) and *sumHBCD* of three hexabromocyclododecane congeners (HBCD-alpha, beta and gamma);
- ➢ 18 mycotoxins: fumonisins B1 and B2 (FB1, FB2), ochratoxin A, B and patuline (OTA, OTB, Pat), trichothecenes from group A (T2, HT2, DAS, MAS) and group B (NIV, DON, DOM1, DON3, DON15, FusX), zearalenone (Zea) and its metabolites (Azea, Azee);
- ➢ phytoestrogens: enterolactone, resveratrol, and two sums: *som_equol* for the sum of 6 isoflavones (biochanin A, daidzein,