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Integrating Selection and Risk Assessment of Chemical Mixtures: A Novel Approach Applied to a Breast Milk Survey

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Background: One of the main challenges of modern risk assessment is to account for combined exposure to the multitude of various substances present in food and the environment.

Objective: The present work proposes a methodological approach to perform chemical risk assessment of contaminant mixtures across regulatory silos regarding an extensive range of substances and to do so when comprehensive relevant data concerning the specific effects and modes of action of the mixture components are not available.

Methods: We developed a complete step-by-step approach using statistical methods to prioritize substances involved in combined exposure, and we used a component-based approach to cumulate the risk using dose additivity. The most relevant toxicological end point and the associated reference point were selected from the literature to construct a toxicological threshold for each substance.

Discussion: By applying the proposed method to contaminants in breast milk, we observed that among the 19 substances comprising the selected mixture, $\sum$DDT, $\sum$PCBi, and arsenic were main joint contributors to the risk of neurodevelopmental and thyroid effects for infants. In addition, $\sum$PCCD/F contributed to the thyroid effect and $\sum$aldrin-dieldrin to the neurodevelopmental effect. Our case study on contaminants in breast milk demonstrated the importance of crossing regulatory silos when studying mixtures and the importance of identifying risk drivers to regulate the risk related to environmental contamination. Applying this method to another set of data, such as human biomonitoring or in ecotoxicology, will reinforce its relevance for risk assessment. https://doi.org/10.1289/EHP8262

Introduction

Human populations are exposed daily through the environment and diet to mixtures of chemicals that can cause adverse health effects. Combinations of stressors can amplify the effects of single stressors and lower the doses required for individual toxic stimuli to cause biological damage (Kostoff et al. 2018). Due to the complexity and multiplicity of mixtures, as well as the various possible routes of exposure, the associated risk is difficult to characterize. Over the past decade, considerable efforts have been made to propose concepts, methods, guidance, and applications for the risk assessment of mixtures (Beronius et al. 2020; Boobis et al. 2008; EFSA 2007, 2008; Fischer et al. 2020; Fox et al. 2017; Meek et al. 2011; WHO 2009b). Two types of approaches were recently developed (EFSA 2013a, EFSA Scientific Committee 2019). The first is the whole-mixture approach, which evaluates the mixture as a single chemical substance. However, it requires information on the dose–response of the whole mixture, which is generally not available except in a few specific cases. The second is the so-called component-based approach, which is built on the available information on exposure and effect data of its individual components. This approach can be used by considering addition of doses, addition of effects, or interactions. The component-based approach is the most commonly applied method and has resulted in the development of several metrics to calculate the risk related to mixtures (EFSA 2019; Kortenkamp et al. 2009; Meek et al. 2011; Sarigiannis and Hansen 2012; WHO 2009b). Most of these metrics are based on dose addition, which is assumed to be conservative (EFSA 2013a, EFSA Scientific Committee 2019; Meek et al. 2011; SCHER 2012; WHO 2009b). Under this assumption, the metrics range from the hazard index (HI) based on health-based guidance values (HBGVs) as a tier 0 in data-poor situations, to the reference point index (RPI), also called the point of departure index (PODI) based on reference points of a specific effect using a common uncertainty factor as a tier 1, and to relative potency factors (RPFs) considering common modes of action and uncertainty factors in a tier 2 data-rich situation (EFSA Scientific Committee 2019; Meek et al. 2011). Recently, Vejdovszky et al. (2019) adapted an RPI for contaminants, creating a modified RPI (mRPI), making it possible to use specific uncertainty factors related to a specific effect. However, the selection process for the specific studied effects and the mixture components were not discussed.

Given the multitude of possible combinations, one major challenge of the component-based approach is to select the substances to be assessed together in a mixture. One frequently used approach is to consider chemicals belonging to the same chemical family (dioxins, organophosphates, etc.) or regulatory silos, which are based on uses (plant protection products, biocidal products, pharmaceuticals, cosmetics, food and feed additives, household chemicals, or industrial chemicals) or media (chemicals in indoor and outdoor air; marine, food, fresh, and groundwater; soils; and sediments), and to compose mixtures by toxicological effects on target organs. This approach is the hazard-wise method, proposed by the

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European Food Safety Authority (EFSA), based on "common adverse outcomes" to place pesticides in cumulative assessment groups (CAGs) (EFSA 2013c; Nielsen et al. 2012; Wolterink et al. 2013). However, in real life conditions, consumers are exposed to substances from different regulatory silos (Evans et al. 2016; Kortenkamp and Faust 2018). Therefore, combined risk assessments of chemicals should not be based solely on regulatory considerations or toxicological properties but should also take into account population combined exposure. Exposure can be estimated by combining consumption patterns or habits of individuals in a population with occurrence levels of chemicals in the media, or by using biomonitoring data (U.S. EPA 2019). The number of combinations of compounds to which an individual in a population is exposed can be large. As a result, statistical methods have been proposed to extract from combined exposure the most relevant mixtures to which a population is exposed (Crépet et al. 2013b, 2019; Traoré et al. 2016). This approach makes it possible to prioritize mixtures for risk assessment and for toxicological and epidemiological studies (Crépet et al. 2013a; Traoré et al. 2018). Another challenge of mixture risk assessment is that among the variety of specific effects that mixture components can cause, it is necessary to prioritize the most relevant ones by considering the population of interest and collecting relevant toxicological information to perform risk assessment.

We propose a methodological approach to perform chemical risk assessment across regulatory silos when relevant comprehensive data concerning specific effects and modes of action (MoAs) of the mixture components are not available. This integrated step-by-step approach draws on the latest methods developed in the field of chemical mixtures to select mixture components and relevant toxicological effects and to estimate the associated cumulative risk. As a case study, the developed method is applied to the French ContaLait survey (Rigourd 2015) of chemicals in breast milk to study the associated risk for breastfed infants. This work presents a novel approach to deal with risk assessment of complex mixtures and to analyze original data on contamination of breast milk.

Integrated Method

To assess the risk of chemical mixtures, we propose application of an integrated approach composed of the following steps (Figure 1).

Step 0: Before Starting the Risk Assessment

This part aims to provide a number of pointers for practical issues commonly encountered when working with data sets for risk assessment. One issue is the analytical quality of the available contaminant concentration or biomonitoring data and the presence of censored data [i.e., when the concentrations were reported below the limit of reporting (LR)]. EFSA (2010) proposed a substitution method with three options: the lower bound (LB) option, which replaces censored data by zero when nondetected and by the limit of detection when nonquantified, the upper bound (UB) and the middle bound (MB) options, which replace censored values by LR or LR/2, respectively. More advanced parametric and nonparametric methods can be applied in regard to risk estimates obtained with substitution options, the sample size, and the censoring percentage. Regarding mixture selection from combined exposure, the LB option has the advantage of prioritizing substances on the basis of real quantified data, whereas with UB and MB the mixtures mostly depend on the LR values, especially in case of high numbers of censored data (Béchaux et al. 2013).

Another issue concerns preliminary grouping of substances and metabolites with very close physicochemical and toxicological properties (lipophilicity, metabolism, toxicokinetics, mechanism of action). These substances can be summed, possibly using toxic equivalent factors (TEFs) when available or molecular weights as proposed for example by Van den Berg et al. 2006 for dioxins and furans and by Nougadère et al. 2014 for pesticides.

Another recurrent question specific to dietary exposure is whether to consider the analyzed nutrients in the risk assessment. Clearly, some nutrients at higher doses than the nutritional recommendations may cause adverse effects and can be integrated into a risk assessment. The choice mostly depends on the problem formulation stage.

Step 1: Mixture Selection from Combined Exposures

Step 1 aims to reduce the number of substances identified in the initial data set by selecting the most prevalent combinations of substances regarding the combined exposures. This step results in the prioritization of the most relevant mixtures to be studied in the subsequent steps. This prioritization is of particular interest when the number of substances identified in the initial data set is large and hazard data are not available for all considered substances. Different statistical methods analyzing the dependencies between exposures to several substances have been developed in recent years to extract prevalent chemical combinations (Kapraun et al. 2017; Su et al. 2014) and to define population clusters based on their combined exposure to chemical mixtures or based on their specific diet in connection with exposure (Béchaux et al. 2013; Traoré et al. 2016). Before applying statistical methods to heterogeneous sets of substances, which could lead to high variability of estimated exposure between substances, it is often necessary to standardize the data sets by dividing each value by the standard deviation of the substance. Generally, several mixtures are obtained from statistical methods, and thus a second mixture selection step is required to prioritize the most relevant ones. Several criteria can be used to select this final mixture: substances with high exposure for one or several clusters of the population (Crépet et al. 2013a, 2013b), specific combinations accounting for a high proportion of the variance of the overall data set [(Crépet et al. 2019) and case study and discussion sections], presence in several clusters of the population (Crépet et al. 2013a, 2013b, and case study and discussion sections), and importance of the mixture regarding toxicity [common effect; carcinogenic, mutagenic, reprotoxic (CMR) classification; EFSA CAG for pesticides (Alarcan et al. 2020), etc.].

Step 2: Selection of Toxicological End Points and Relevance of Additivity

Step 2.1: Selection of toxicological end points for populations of concern. This step consists of screening the literature on human, animal, and *in vitro* surveys to identify the different potential toxicological effects of the selected mixture components. From this literature review, we recommend selection of the most relevant end points for the studied population from the most representative end points associated with the mixture components and to develop the steps after for these selected toxicological end points. The use of data obtained from recent computational models based on high-throughput screening testing (www.epa.gov) through new approach methodologies (NAMs) for hazard assessment (*in vivo*, *in vitro*, organoids, *-omics*, and *in silico*) can be of help from a mechanistic point of view because they span different layers of biological organization.

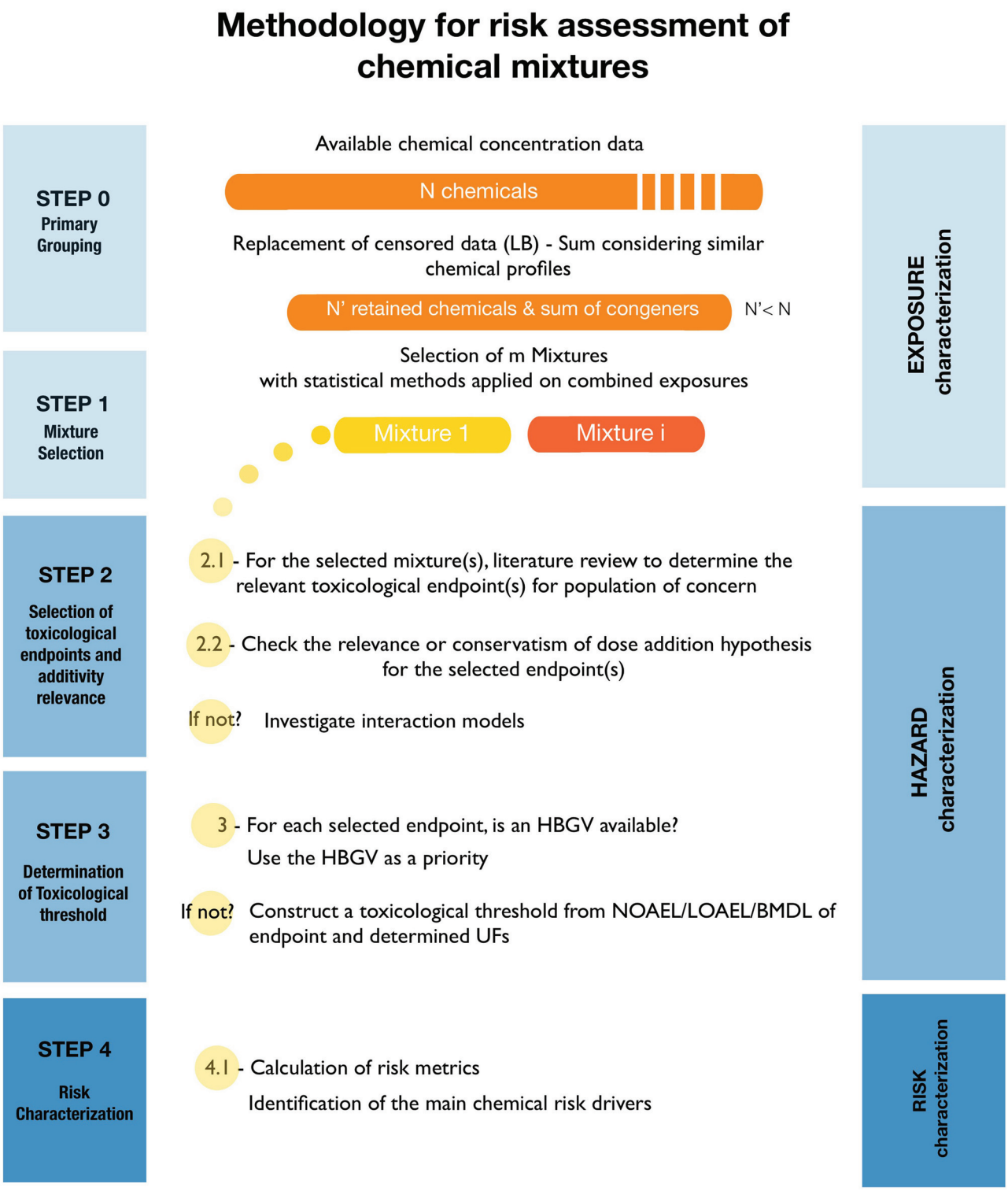


Figure 1. Integrated approach to assess the risk of chemical mixtures. Note: BMDL, benchmark dose lower bound; HBGV, health based guidance value; LB, lower bound; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; UF, uncertainty factor.

Step 2.2: Relevance of dose additivity for mixture components. Based on scientific literature comparing the combined effects induced by exposure to multiple substances with similar and independent (dissimilar) actions (Altenburger et al. 2013; Backhaus and Faust 2012; EFSA 2013a, 2013b, 2019; Faust 2003, 2001; Jonker et al. 2004; Junghans et al. 2006; Kortenkamp et al. 2009), dose addition was established as the default hypothesis recommended by several institutions for mixture risk assessment (EFSA 2013a, 2019; Meek et al. 2011; SCHER 2012; WHO 2009b). Importantly, the dose-addition approach estimates the combined effects of substances with similar actions and ensures adequate conservatism in the case of independent actions, because it tends to overestimate the combined effects (Faust 2003). Moreover, at low exposure doses [i.e., around the no observed adverse effect level (NOAEL)], it was observed that toxicologically relevant interactions were uncommon (EFSA Scientific Committee 2019). The

dose-addition approach also applies when no information is available on the MoA or dose–response relationship.

Therefore, to investigate the dose-addition hypothesis, we propose to first verify whether exposure levels correspond to low doses (i.e., around the NOAEL). If not, we recommend screening the literature to study the mode or mechanism of action of mixture components and to find out whether results are available on the dose–response of similar mixtures.

In the case of results showing greater- or less-than additive responses (i.e., interactions), adapted methods taking into account such interactions can be investigated. The use of extra uncertainty factors derived from interaction data at high doses (Ragas et al. 2010) or the interaction-based HI (Wilbur et al. 2004; U.S. EPA 2000, 2007; Mumtaz and Durkin 1992; Sarigiannis and Hansen 2012) are some of them.

Step 3: Toxicological Thresholds (TTs)

Once toxicological end points were selected and dose additivity investigated, we propose to perform for each substance a second round of literature review to extract the toxicological values associated with each selected end point. The institutional HBGV, when based on the selected end point, can be used as a priority. If such an HBGV is not available, we propose building a TT for each selected end point from existing data. To this end, the BMDL, the NOAEL, or by default the lowest observed adverse effect level (LOAEL), obtained from epidemiological human studies or *in vivo* experiments, can be used as a reference point (RP) to derive a TT in applying uncertainty factors (UFs). One or several UFs are thus applied to make it possible to account for sensitivity difference between individuals (intraspecies variability), animals and humans (interspecies variability), adults and infants, or lack of data, among other factors. Accepted default values for UFs have been established by the WHO (2011) and EFSA (2012). Recently, Vejdovszky et al. (2019) presented a detailed decision tree to apply UFs in the context of cumulative risk assessment of food contaminants.

A careful literature review is important to select relevant studies of high quality on models close to the concerned population and preferentially with similar exposure conditions (e.g., period, route, and duration). When no RP for the selected end point can be found for a data-poor substance, we suggest using in the following order of priority: *a*) the value obtained for another effect related to the studied end point; *b*) the value of an analog compound for the studied effect; or *c*) the HBGV established for the most sensitive effect.

Step 4: Component-Based Risk Assessment under Dose-Addition and Uncertainty Analysis

Several component-based risk metrics related to dose addition have been published (EFSA 2013a; EFSA Scientific Committee 2019; Kortenkamp et al. 2009; Meek et al. 2011; Sarigiannis and Hansen 2012; Vejdovszky et al. 2019; WHO 2009b, 2017). To select one of them, we propose to consider the heterogeneity of the collected data (human, animal, *in vitro*), heterogeneity of UF values, and the type of collected end points (most sensitive effect, specific effect, mode of action, etc.). For example, in the case of a CAG established by EFSA for a specific effect of pesticides on the basis of animal experiments, for a substance s, a relative potency factor (RPF_s) can be calculated by dividing the NOAEL of the index compound ($NOAEL_{index}$) by the NOAEL of the substance s ($NOAEL_s$): $RPF_s = NOAEL_{index}/NOAEL_s$. Then, the exposures of each substance s (E_s) among the S substances in the mixture can be summed to cumulate the exposure: $CumE = \sum_{s=1}^{S} E_s x RPF_s$ and to estimate the margin of exposure $MOE = NOAEL_{index}/CumE$. This approach is only possible when homogeneous toxicological data for a specific effect are available, leading to a single UF for all the mixture components. In similar conditions with a single UF, the RPI, also called the point of departure index (PODI), can be applied. The RPI is the sum of the exposure (E_s) to each component s divided by the RP (RP_s). This sum is equivalent to the inverse of the sum of the margin of exposure (MOE) of each single component, named the combined margin of exposure (MOET):

$$RPI = PODI = \sum_{s=1}^{S} \frac{E_s}{RP_s} = \cdots = \sum_{s=1}^{S} \frac{1}{MOE_s} = \frac{1}{MOET}.$$

These metrics were modified to consider different UFs between substances. Therefore, the mRPI (Vejdovszky et al. 2019) or adjusted point of departure index (PODIadj) (WHO 2020) and normalized combined margin of exposure (nMOET) (Sprong et al. 2020) were developed. The HI is the sum of the hazard quotients (HQs) of individual components s: $HQ_s = E_s/HBGV_s$. HI can be used in a tier 0 approach to calculate the risk based on the RP from the most sensitive effect and the associated UF of each individual component.

Once risk results are obtained, we propose characterizing the sources of uncertainty associated with the different steps (censored data hypothesis, mixture choices and components, dose-additivity hypothesis, etc.), and if needed to analyze the impact on the risk of different scenarios (EFSA 2017a; IPCS-WHO 2008, 2014; NRC 2013).

Case Study: Risk Assessment of Chemical Mixture in Breast Milk

The ContaLait Survey

The ContaLait survey (Rigourd 2015; Vacchina et al. 2017) is a French study of contaminants in breast milk, led by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) and the Assistance Publique des Hôpitaux de Paris (APHP), with the participation of the Laboratory for the Study of Residues and Contaminants in Foods (LABERCA/ONIRIS), the Laboratory of Food Security (LSA/ANSES) and the laboratory of Ultra Traces Analysis Aquitaine (UT2A). The aim of the ContaLait survey was to assess breast milk contamination in women in France. Breast milk samples from 180 mothers were collected at 6 milk banks in France between 2013 and 2015. All participants gave their written informed consent. Selected participants were healthy mothers over 18 years of age, living in France, breastfeeding a healthy child no older than 6 months of age, and having donated their milk to a milk bank. Donors provided a pool of breast milk samples collected at home over a period of 2–3 wk. Milk samples from a single mother were pasteurized at 62.5°C for 30 min, cooled down at 40°C, and then frozen at −20°C before analysis. The breast milk samples used in this work were the ones declared unsuitable for consumption by newborns due to a total aerobic flora after 24 h incubation at 37°C $\geq 10^6$ bacteria per milliliter. Analyzed substances were selected from a first estimate of the risk calculated using published data. For that, breast milk contamination data were searched using the search engine PubMed and the following keywords "contaminated breastmilk" or "breastmilk contamination," or "name of substance AND contaminated breastmilk," or "name of substance AND breastmilk contamination." The names of the substances were the ones targeted in the infant French Total Diet Study (Hulin et al. 2014). From the 131 obtained surveys, we selected human studies conducted from 2000 to 2011 in Europe, North America, Japan, and Australia, and with at least 30 subjects. For the 79 selected studies we reported the following

information: the date, the country, the number of women with their age, body weight (BW), number of children, dietary and smoking habits, the sampling period, the sample size, the analytical method, and the quantity taken. All information on the distribution of contamination was also collected (mean, median, standard error (SE), minimum, maximum, P95th percentiles). The mean and the P95th (or when not available the mean+2 × SE) were combined with the mean and the P95th of the breast milk consumption data from the *Child-Specific Exposure Factors* handbook (U.S. EPA 2008) to assess the theoretical exposure for breastfed 1-month-old children. Exposures were compared to available HBGVs, and priority was given to substances exceeding the HBGVs in at least one study. In all, 107 minerals and substances from different chemical families potentially present in breast milk were analyzed: PCBs, dioxins, organochlorine pesticides, metals, and nutrients (Excel Table S1).

Application of the Integrated Method to Breast Milk Contamination

Following the described steps in the previous section, we applied the integrated method to the French ContaLait survey (Rigourd 2015) to perform risk assessment for chemical mixtures in breast milk.

Step 0: Analyzed substances and sum of congeners with similar chemical profiles. We first removed all nutrients to focus on chemical risk assessment. We then summed the concentrations of the substances with very close chemical structures or similar mechanisms/modes of action as follows. For dioxins and furans, we used toxic equivalent quotients (TEQs) from the WHO 2005 (Van den Berg et al. 2006) (Excel Table S1). For pesticides, metabolites, closely related substances, and/or isomers, we used coefficients corresponding to the ratios of molecular weights (MW) of analogs (i.e., MW dieldrin/MW aldrin) and of parent/metabolite (i.e., MW DDT/MW DDE) (Fréry et al. 2017; Nougadère et al. 2014):

- Σaldrin-dieldrin = 1.04 aldrin + dieldrin
- Σchlordane-nonachlor = alpha-chlordane + gamma-chlordane + 0.97 oxy-chlordane + 0.92 *cis*-nonachlor + 0.92 *trans*-nonachlor
- ΣDDT/E/D = p,p′ − DDT + o,p′ − DDT + 1.1 × p,p′ − DDE + 1.1 × p,p′ − DDD
- ΣHCH = alpha-HCH + beta-HCH + delta-HCH
- Σheptachlor = heptachlor + *cis*-epoxide + *trans*-epoxide.

For the following substances, we summed the concentrations without using a coefficient, in line with current practice (Rivière et al. 2019; van den Berg et al. 2017):

- ΣPCBi = PCB28 + PCB52 + PCB101 + PCB138 + PCB153 + PCB180 (Van den Berg et al. 2017)
- ΣPBDEs = PBDE28 + PBDE47 + PBDE99 + PBDE100 + PBDE153 + PBDE154 + PBDE183 except PBDE 209 which has specific toxicokinetics and was considered separately (Rivière et al. 2019)
- ΣPBBs = PBB52 + PBB101 + PBB153 (ANSES 2016; IARC 2013)
- ΣHBCDDs = alpha-HBCD + beta-HBCD + gamma-HBCD (Rivière et al. 2019).

To focus on chemicals with observed concentration data, we used the LB scenario and replaced censored data by zero. This approach resulted in null concentrations for all individuals for 16 substances that were removed. We obtained a final data set containing 32 substances or sum of substances (Excel Table S1).

Step 1: Mixture selection for exposure through breast milk. **Breast milk exposure estimates.** We estimated exposure to the $s = 32$ selected substances for 180 infants using consumption and standard BW values proposed by EFSA (2017b) for infants under 6 months of age. To this end, we multiplied each concentration C_s in the breast milk by a mean consumption of 763 mL and divided by a mean BW of 6.1 kg:

$$E_s = \frac{C_s \, x \, 0.763}{6.1}.$$

The combined exposure expressed in micrograms per kilogram BW per day formed a matrix E of 32×180 size.

Statistical method to define mixtures from combined exposure. We applied the sparse nonnegative matrix underapproximation (SNMU) (Gillis and Plemmons 2013) to the exposure matrix E to define mixtures from the 32 substances in the ContaLait data set. The SNMU solution approximates the nonnegative matrix E by two nonnegative matrices W and H with lower dimensions, $(s \times k)$ and $(k \times n)$, respectively, such that the product of the two is as close as possible to the original matrix. In short, s represents the number of substances, n the number of individuals, and k the preset number of mixtures (Traoré et al. 2018). The matrices W and H were obtained by minimizing the criterion: $\|E - \mathrm{WH}\|^2$, such that $W \geq 0$ and $H \geq 0$. Matrix W contains weights of substances per mixture, and matrix H contains the coefficients of the presence of the mixture per individual or exposure day. The nonzero entries in each column of W indicate the components of each mixture k. The higher the W weight, the higher the participation of the substance in the mixture. The nonzero entries in each column of H indicate the mixture to which the individual was exposed. The higher the H weight, the higher the contribution of the mixture to the whole exposure.

Mixture selection by coupling statistical criteria with the relevance of combined exposure profiles and mixture composition. The selection of studied mixture(s) was performed by first selecting candidate values for the optimal choice of k, and second by examining results of hierarchical clustering applied to the H matrices calculated with the different candidate values (Gazan et al. 2016; Traoré et al. 2016; Traoré et al. 2018). Candidate values were selected by running the SMNU algorithm for several values of k comprised between 1 and K, and calculating the associated residual sum of squares between the exposure matrix E and the product of the estimated matrices W and H. Then, the different k values for which a substantial decrease in the residue values was observed in comparison with those with k-1 were considered candidate values. Hierarchical clustering was then applied to each matrix H obtained with the candidate values to group individuals with similar profiles of exposure to the obtained k mixtures. Further, we examined how exposure to the k mixtures characterized the clusters. The k values for which at least one mixture was not used to characterize a cluster, or concerned only a small part of the population, were rejected. Once the optimal value of k was defined, the relevant mixtures were prioritized regarding the number of individuals exposed, the level of exposures of each individual group, and the mixture components.

Step 2: Selection of toxicological end points and relevance of additivity. **Step 2.1: Selection of toxicological end points.** To select toxicological end points, we scrutinized the potential effects using the search engine PubMed and generic names of substances or substance families (aldrin or dieldrin, arsenic, chlordane or nonachlor, dichlorodiphenyltrichloroethane, dioxin, heptachlor, hexachlorobenzene, hexachlorocyclohexane, mercury, methylmercury, mirex, pentachlorobenzene, perfluorated and polybrominated biphenyls, polybrominated diphenyl ethers, polychlorobiphenyl) as key words, up to December 2017. The search was assessed using the keywords "name of substance AND toxicity," or "name of substance AND toxicity AND end point." Selected end points such as reproduction, carcinogenesis, hepatic, immune, thyroid, nervous system, and development were of particular interest and were reported for all the substances. The sum of publications addressing these end points represented more

than 50% of total publications on toxicity for a majority of substances (Excel Table S2 and Figure S1). Thyroid, neural, and developmental effects, which are of particular concern for newborns, were largely reported for almost all substances (Excel Table S3, Figure S2). We decided to focus on nervous system development; thus thyroid and neurodevelopmental effects were selected as end points for the reasons described in the next paragraph. The search focusing on these two end points was performed for each substance up to December 2019. In parallel with this selection, effects for which institutional HBGVs have been established were screened from the reports and databases of ATSDR (2002, 2007, 2017); U.S. EPA (1992, 2012); WHO (2003); EFSA (2012); and ANSES (2007). It was found that the available HBGVs were established on diverse critical effects, hindering the selection of a common toxicological effect.

Regarding the targeted population of children, we selected the thyroid and neurodevelopmental. Thyroid disruption may underlie certain neurodevelopmental alterations because thyroid hormones play a vital role in brain development (Williams 2008; Zoeller and Rovet 2004). They regulate several processes, such as neuronal proliferation and migration or myelination (Zoeller and Rovet 2004). In humans, the thyroid gland of the fetus begins to function only from the 18th to the 20th week of pregnancy. Before this period, the fetus is dependent on maternal thyroid hormones, which regulate neural development even after the 20th week of pregnancy (Morreale de Escobar et al. 2004; Zoeller and Rovet 2004). Thus, any change in maternal hormone levels during the prenatal period is likely to have adverse effects on brain development and cognitive abilities (Thompson et al. 2018). Because the nervous system continues to develop in the neonatal and postnatal periods, hypo- or hyperthyroidism may have consequences in newborns (Morreale de Escobar et al. 2004). Experimental studies using rodents show similar regulations by thyroid hormones of cerebral development during the prenatal and postnatal periods (for reviews, see Morreale de Escobar et al. 2004; Zoeller and Rovet 2004). As in humans, the fetal thyroid gland is inactive during the first third of gestation, with a requirement for maternal sources, and thereafter both fetal and maternal hormones are necessary for normal cerebral development (Prezioso et al. 2018).

Step 2.2: Relevance of dose additivity for thyroid and neurodevelopmental effects. To evaluate the relevance of dose additivity for thyroid and neurodevelopmental effects, we used the keywords "mixture AND thyroid and rodents" and performed the search in PubMed up to December 2019. Further details are described in Supplemental Material, "S1. Search and evaluation strategy on relevance of dose-additivity." Through this search, we found that the majority of selected substances have been shown to induce thyroid effects and, in particular, *in vivo* changes in thyroid hormone levels in experimental and human studies. Furthermore, analysis of previous experimental studies comparing the effects of separate and combined exposure to chemicals, similar to those present in the selected mixture, showed dose additivity. Of the four selected studies comparing the effects of separate and combined developmental exposure to at least two different substances of the studied mixture (Crofton et al. 2005; Flippin et al. 2009; Miller et al. 2012; He et al. 2011), three investigated the nature of interaction between the substances of the mixtures. Crofton et al. (2005) reported that postnatal exposure of rats to a mixture of TCDD and PCBs reduced thyroxin (T4) levels in an additive manner at low doses; these effects were predicted by a self-generated model approach. Thyroxin or T4 is the precursor of biologically active T3 and the main released hormone in the blood. Similar additive effects on T4 levels have been described for postnatal or perinatal exposure to mixtures of TCDD/PCBs/pesticides (Flippin et al. 2009) or PCBs/PBDEs in the rat (Flippin et al. 2009; Miller et al. 2012). Concerning neural effects, the search using the keywords "mixture AND nervous system (or brain) and rodents" focused on *in vivo* studies using at least two different substances present in the studied mixture and neurodevelopmental or behavioral effects (See also Supplemental Material, "S1. Search and evaluation strategy on relevance of dose-additivity"). Of the seven selected studies (Eriksson et al. 2006; Fischer et al. 2008; Gralewicz et al. 2009; He et al. 2011; Reardon et al. 2019; Roegge et al. 2004; Sitarek and Gralewicz 2009), three investigating the nature of interaction between PCBs and MeHg (Roegge et al. 2004), PCBs and PBDEs (Eriksson et al. 2006), or PBDEs and MeHg (Fischer et al. 2008) suggested additive effects on motor coordination. Altogether, these observations led us to use dose additivity to predict the mixture effects on the thyroid and neurodevelopment.

Step 3: TTs for thyroid and neurodevelopmental effects. We conducted a new literature review for each substance of mixture 1 to collect toxicity data related to the thyroid and neurodevelopmental end points using the PubMed search engine up to December 2019. For thyroid effects, the keywords "name of substance AND thyroid" were used. The data reporting effects on thyroid hormone levels were chosen primarily to *a*) have a homogeneity in the effects compared between the studied substances and *b*) establish a link between the thyroid and nervous systems. For neurodevelopmental effects, the keywords "name of substance AND nervous system," "name of substance AND brain," or "name of substance AND neurodevelopment" were used. It was decided to focus on neural functions and behaviors known to be regulated by thyroid hormones (Zoeller and Rovet 2004), such as cognitive function (learning and memory), sensorimotor development, and motor behavior/locomotor activity. Because the targeted population was infants exposed through breast milk, epidemiological and experimental *in vivo* studies focusing on exposure periods of gestation and/or lactation and/or postnatal exposure were first selected. When data on these developmental exposure periods (gestation, lactation, postnatal periods, and prepubertal/pubertal periods) were unavailable, studies on adult exposures were considered, and the different sensitivity between adults and infants was accounted for in the UFs.

We prioritized the available HBGVs for the considered end points (thyroid and neurodevelopmental effects) from the reports and database of ATSDR 2002, 2007, 2017; U.S. EPA 1992, 2012; WHO 2003; EFSA 2012; and ANSES 2007. HBGVs were taken as such without any change of the UFs applied for their determination. Otherwise, we used the results of the literature search, as described above in step 3, to identify the key experimental studies that made it possible to construct toxicological thresholds (TT) based on each of the two selected end points. Particular attention was paid to the experimental studies addressing exposure effects either on thyroid hormone levels or sensorimotor or motor and behavioral responses as a priority during gestational and/or lactational periods of exposure. From the identified studies, the NOAEL or LOAEL doses for the selected effects were retained (Tables 1 and 2). Species, exposure period, and duration were also collected to define appropriate UFs (Tables 1 and 2).

Several UFs were multiplied to determine the final UF, which was then used to calculate the TT (Tables 1 and 2). A UF of 10 was generally used to adjust interspecies variability between animals and humans, whereas a UF of 3 was applied when a PBPK model was used (IPCS 2009; WHO 1994, 2009a); another UF of 10 was used to adjust intraspecies variability; a supplementary UF of 3 or 10 (the default value) was applied to extrapolate NOAEL from LOAEL values (Hasegawa et al. 2010; Pohl and Abadin 1995); and a UF of 3 was used to account for infant sensitivity in comparison with adult individuals (Hasegawa et al.

Table 1. Details of key studies, specific effects, reference points, uncertainty factors, toxicological thresholds (TT) and health-based guidance values (HBGV) for the thyroid effect.

				Experimental study					TT	HBGV
Substance	Reference	Effect	Epidemiological study	Species	Exposure period	Exposure duration	Reference point (μg/kg/d)	Uncertainty factor	(μg/kg/d)	
Arsenic (As)	Sun et al. 2017	Juvenile females were exposed to arsenate (AsV) or arsenite (AsIII) at 10 or 100 μg/L in drinking water for 8 wk. Exposure for 4–8 wk disrupted T4 homeostasis and influenced the related gene transcription from 10 μg/L and damaged the thyroid gland at 100 μg/L.	—	Balb/c mice	PND21–PND77	Repeated doses for 8 wk	LOAEL = 1.5	300 [Interspecies: 10, Intraspecies: 10, LOAEL → NOAEL: 3]	0.005	—
ΣChlordane-nonachlor	Bondy et al. 2004	Male and female rats were exposed through food to trans-nonachlor at 0.5, 1.25, or 5 mg/kg/d. Exposure increased T4 levels in a dose-dependent manner in females, with a significantly 2-fold increase at the highest dose.	—	Sprague-Dawley rats	End of puberty–adulthood	Repeated doses for 90 d	NOAEL = 1250	300 [Interspecies: 10, Intraspecies: 10, adult → infant: 3]	4.2	—
Dichlorodiphenyl-trichloroethanes/dichloroethylene/dichloroethane (ΣDDT/D/E)	Yaglova et al. 2016, 2018	Rats were exposed from PND1 until 6 weeks of age (through milk of treated dams, then drinking water) to DDT at 3.7 μg/kg. Reduced T4 levels in males and females, reduced T3 levels in females as well as increased TSH levels in both males and females were observed.	—	Wistar rats	PND1 to 6 weeks of age	Repeated doses for 6 or 10 wk	LOAEL = 3.7	300 [Interspecies: 10, Intraspecies: 10, LOAEL → NOAEL: 3]	0.012	—
Hexachlorobenzene (HCB)	Hadjab et al. 2004	Adult Sprague-Dawley male rats were orally exposed to HCB at 0.16, 4, or 16 mg/kg/d. Exposure reduced levels of total T4 from the dose of 4 mg/kg/d (Hadjab et al. 2004).	—	Sprague-Dawley	Adulthood	Repeated doses for 4 wk	NOAEL = 160	300 [Interspecies: 10, Intraspecies: 10, adult → infant: 3]	0.53	—
Methylmercury (MeHg)	EFSA 2012	Studies of the Faroe Islands cohorts indicated an association between prenatal exposure and auditory function, one of the thyroid regulated processes during development (Grandjean et al. 1997; Murata et al. 2004).	7- to 14-y-old children of the Faroe Islands cohorts generated during 1986–1987	—	—	—	NOAEL = 1.2 Hg	6.4 [Intraspecies: 3.2, lack of data: 2]	—	0.19 μg Hg
Mirex	U.S. EPA 1992	Splenic fibrosis and cystic follicles of the thyroid were seen in male rats at and above 0.7 mg/kg/d. Based on liver and thyroid effects, this study defined a NOAEL of 0.07 mg/kg/d and a LOAEL of 0.7 mg/kg/d (McDonald 1991).	—	F344/N rats	Puberty–Adulthood	Repeated doses for 104 wk	NOAEL = 70	300 [Interspecies: 10, Intraspecies: 10, lack of data: 3]	—	RfD = 0.2
Polybrominated biphenyls (ΣPBBs)	Werner and Sleight 1981	Swine dams were exposed to a Fire-Master PB-6 mixture during the gestational and early lactational period. Reduced serum levels of T3 and T4, and thyroid hyperplasia were observed in 4-wk-old offspring at 2.5 and 1.25 mg/kg/d. A NOAEL dose of 0.125 mg/kg/d was reported.	—	Sow	GD8–PND4	Repeated doses for 12 wk	NOAEL = 125	100 [Interspecies: 10, Intraspecies: 10]	1.25	—
Polybrominated diphenyl ethers (ΣPBDEs)	ATSDR 2017	Exposure to PBDE-99 at 60 μg/kg/d and 300 μg/kg/d by gavage reduced T4 levels in dams at postgestational day 1. The lowest dose reduced free T4 levels in female offspring at PND1. The higher dose reduced T4 and FT4 levels in female offspring and T4 levels in male offspring at PND22 (Kuriyama et al. 2007).	—	Wistar rats	GD6	Single dose	LOAEL = 60	1,000 [Interspecies: 10, Intraspecies: 10, LOAEL → NOAEL: 10]	—	MRL acute = 0.06
PBDE-209	Wang et al. 2019	Male rats were orally exposed to BDE-209 at 5, 50, and 500 mg/kg/d. Exposure reduced total and free T4 levels, and total T3 levels at the highest dose. Free T3 levels were reduced from the dose of 50 mg/kg/d.	—	Sprague-Dawley rats	Adulthood	Repeated doses for 28 d	NOAEL = 5000	300 [Interspecies: 10, Intraspecies: 10, adult → infant: 3]	16.7	—

Table 1. (*Continued.*)

Substance	Reference	Effect	Epidemiological study	Experimental study			Reference point (μg/kg/d)	Uncertainty factor	TT	HBGV
				Species	Exposure period	Exposure duration			(μg/kg/d)	
Indicator polychlorinated biphenyls (ΣPCBi)	Provost et al. 1999	Dams were exposed through food to Aroclor 1254 at 0.1 or 1 mg/kg/d. Offspring had reduced T3 levels for both doses on PND30, and reduced T4 levels on PND15 and PND30 in rats exposed to the higher dose.	—	Sprague-Dawley rats	GD0–PND15 or GD0–PND30	Repeated doses	LOAEL = 100	1,000 [Interspecies: 10, Intraspecies: 10, LOAEL → NOAEL: 10], ∑PCB → ΣPCBi: 2	0.05	—
Dioxins and furans (ΣPCDD/Fs)	U.S. EPA 2012	Increased TSH levels in newborn infants born to mothers exposed to 2,3,7,8-TCDD after the 1976 Seveso accident (Baccarelli et al. 2008).	Newborns: study conducted between 1994 and 2005	—	—	—	LOAEL = 0.000021	30 [Intraspecies: 3 LOAEL → NOAEL: 10]	—	RfD = 0.0000007
Pentachlorobenzene (PeCB)	McDonald 1991	Animals were exposed to 2.2 to 164 mg/kg/d in rats and 5.2 to 410 mg/kg/d in mice for 13 wk. Exposure to PeCB reduced free thyroxin and total thyroxin levels in males and females.	—	F344 rats and B6C3F1 mice	Adulthood	Repeated doses for 13 wk	LOAEL = 2400	900 [Interspecies: 10, Intraspecies: 10, LOAEL → NOAEL: 3, adult → infant: 3]	2.7	—
Perfluorooctanesulfonic acid (PFOS)	Lau et al. 2003	Pregnant rats were exposed by gavage to PFOS at 1, 2, 3, 5, or 10 mg/kg/d from GD2 to GD20-21. Decreased levels of T4 and T3 in pregnant dams and suppressed T4 levels in PFOS-treated rat pups were observed.	—	Sprague-Dawley rats	GD2–GD20	Repeated doses	HED = 1.4	30 [Intraspecies: 3 LOAEL → NOAEL: 10]	0.05	—

Note: —, no data available; FSH, follicle-stimulating hormone; GD, gestational day; HGBV, health-based guidance values; HED, human equivalent dose; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; PND, postnatal day; RfD, reference dose; TCDD, Tetrachlorodibenzo-p-dioxin; TT, toxicological thresholds.

2007). When necessary, an extra factor of 2 or 10 for database insufficiency was added when human and animal surveys were used, respectively (IPCS 2009; WHO 1994, 2009a). In the case of short-term exposure (single exposure, for example) occurring during a critical period of development, no supplementary UF was applied, considering that it is sufficient to cause an adverse effect. The TT was thus calculated by dividing the retained NOAEL or LOAEL dose by the final UF.

Step 4: The mRPI as a risk metric. For each infant in the ContaLait survey, we calculated the mRPI proposed by Vejdovszky et al. (2019) by the following equation summing for each substance s, the hazard quotient between the exposure and the TT or the HBGV.

$$mRPI = \sum_{s=1}^{S} \frac{E_s x\, UFs}{RP_s} = \sum_{s=1}^{S} \frac{E_s}{TTs}$$

$$or\, mRPI = \sum_{s=1}^{S} \frac{E_s}{HBGVs}.$$

An mRPI <1 indicates that health risk is considered unlikely.

Results

Mixture selection. Application of SNMU to exposure to the 32 substances using $K = 15$ as a starting number of mixtures led to $k = 8$ and 3 as candidate values for an optimal value of k. The first mixture explained the largest proportion of the total exposure variance (41.2%), whereas mixtures 2 to 7 explained between 3% and 5.4% of the variance and mixtures 8 to 15 less than 3% (Excel Table S4). The reduction of the residual sum of squares using $k = 8$ compared with $k = 3$ was very low. Therefore, it was decided to use $k = 3$ to perform clustering of the population. Clustering of the individuals led to three optimal clusters A, B, and C of infants with different levels of exposure to the first three mixtures (Table 3). Clusters A, B, and C were composed of 55, 91, and 34 individuals, respectively. The infants in the three clusters were all exposed to mixture 1, which contributed to 83.9%, 92.5%, and 47.5% of the overall exposure of clusters A, B, and C, respectively. Clusters B and C, composed of 125 infants in total, presented higher exposures to mixture 1. This mixture was composed of 19 substances. The participation of the 19 substances to the overall exposure to the mixture 1 varied between 0.1% and 14.8% (Figure 2; Excel Table S5). The main substances included in this mixture were hexachlorobenzene, dioxins and furans, the sum of heptachlor, and PCBs. All the substances in mixture 1 were also involved in mixtures 2 and 3, except perfluorononanoic acid (PFNA), which was present only in mixture 3 (Figure 2; Excel Tables S6 and S7). Mixture 2 represented 4.2% of the total variance (Excel Table S4) and was involved in 15.5% of the exposure of cluster A (Figure 2). This mixture was composed of nine substances already present in mixture 1. Mixture 3 accounted for 5.4% of the total variance (Excel Table S4) and concerned mainly the individuals in cluster C (Figure 2). It was composed of four perfluorinated compounds, of a total of seven substances. Because all individuals were exposed to mixture 1, which included 19 substances among the 32 selected substances, mixture 1 was used to perform the cumulative risk assessment for the entire population and for the three clusters.

Retained HBGVs and TTs for thyroid and neurodevelopment. The key studies supporting the selected HBGVs, and the retained reference points and UFs used to construct toxicological thresholds for thyroid or neurodevelopment are presented in Tables 1 and 2, respectively. More details on the studies and choices of the retained values are given in the Supplemental Material, "S2. Study details for defining HBGVs and TTs values."

Table 2. Details of key studies, specific effects, reference points, uncertainty factors, TT, and HBGVs for neurodevelopmental effect.

				Experimental study					TT	HBGV
Substance	Reference	Effect	Epidemiological study	Species	Exposure period	Exposure duration	Reference point (μg/kg/d)	Uncertainty factor	(μg/kg/d)	
ΣAldrin-dieldrin	Smith et al. 1976	Male squirrel monkeys were orally exposed to dieldrin at 10 or 100 μg/kg/d. Exposure to the higher dose affected visual nonspatial successive discrimination reversal task.	—	Monkey (*Saimiri sciureus*)	Adulthood	Repeated doses for 55 d, then post-shift exposure	NOAEL = 10	300 [interspecies: 10, intraspecies: 10, adult → infant: 3]	Vti = 0.03	—
Arsenic (As)	Tsuji et al. 2015	Associations of early-life arsenic exposure and decreased verbal and full scale IQ in preschool-age girls but not boys (Hamadani et al 2011). On the basis of this work, Tsuji et al. (2015) defined an RfD of 0.4 – 1 μg/kg/d.	5-y-old children born between 2002 and 2003	—	—	—	LOAEL = 1.1	6 [intraspecies: 3, extra factor: 2]	0.2	—
ΣChlordane-nonachlor	Cassidy et al. 1994	Dams were exposed during gestation and lactation to technical chlordane at 0.1, 0.5, and 5 mg/kg/d. Their weaned offspring were individually exposed to the same doses. Exposure induced improved spatial performance (Cincinnati Water maze test) and increased auditory startle response in female offspring since the dose of 100 μg/kg/d.	—	Sprague-Dawley rats	GD4-PND21, then PND22–PND80	Repeated doses	LOAEL = 100	300 [interspecies: 10, intraspecies: 10, LOAEL → NOAEL: 3]	0.33	—
Dichlorodiphenyltrichloroethanes/dichloroethylene/dichloroethane (ΣDDT/D/E)	ATSDR 2002	Exposure of 10-d-old male mice to a single dose of technical DDT (0.5 mg/kg; Eriksson et al. 1992) or DDT and its metabolite (0.5 mg/kg and 0.7 mg/kg, respectively; Eriksson et al. 1990) resulted in increased spontaneous motor behavior at the age of 4–5 months and in alterations in muscarinic receptors in the brain.	—	NMRI mice	PND10	Single dose	LOAEL = 500	1,000 [Interspecies: 10, Intraspecies: 10, LOAEL → NOAEL: 10]	—	TDI = 0.5
Hexachlorobenzene (HCB)	Lilienthal et al. 1996	Dams were exposed to HCB at 0.32, 0.64, or 1.280 mg/kg/d. Exposed male offspring showed normal open-field activity on PND21 and active avoidance learning on PND90. On PND150, they exhibited reduced performance in an operant conditioning task.	—	Wistar rats	90 d before mating until offspring weaning	Repeated doses for 132 d	NOAEL = 640	100 [interspecies: 10, intraspecies: 10]	6.4	—
Hexachlorocyclohexanes (ΣHCHs)	Johri et al. 2007	Dams were exposed to lindane at 62.5, 125, or 250 μg/kg. Effects on spontaneous locomotor activity were observed in offspring on 3, 6, or 9 weeks of age for the two higher doses. The sex of analyzed offspring was not indicated.	—	Wistar rats	GD5–GD21	Repeated doses	NOAEL = 62.5	100 [interspecies: 10, intraspecies: 10]	0.62	—
ΣHeptachlor	ATSDR 2007	Dams were dosed with 0.03, 0.3, or 3 mg/kg/d. The righting reflex was delayed in PND2–PND5 female offspring for the higher dose. Offspring of both sexes exposed until PND21 showed altered activity, and those exposed until PND42 displayed altered spatial learning memory in the Morris Water maze (Moser et al. 2001).	—	Sprague-Dawley rats	GD12–PND7, then individual exposure of offspring until PND21 or PND42	Repeated doses	LOAEL = 30	300 [interspecies: 10, intraspecies: 10, LOAEL → NOAEL: 3]	—	MRL = 0.1
Inorganic mercury (inorganic Hg)	Huang et al. 2011	Dams were exposed from premating until weaning and weaned offspring for 7 wk to HgCl2 (500 μg/kg/d). Neurobehavioral defects in developing offspring mice exposed to a low-dose mercuric chloride during maternal gestation and lactation. After weaning: increased locomotor activity, motor equilibrium impairment, and auditory dysfunction.	—	ICR mice	4 wk before mating until weaning (PND21), or after weaning for 7 wk.	Repeated doses	LOAEL = 370	300 [interspecies: 10, intraspecies: 10, LOAEL → NOAEL: 3]	1.23 HgTT	—
Methylmercury (MeHg)	EFSA 2012	Studies of the Faroe Islands (Murata et al. 2004; Grandjean et al. 2007) and Seychelles (Davidson et al. 2008) cohorts indicated an association between developmental exposure and neurobehavioral outcomes.	Children of the Faroe Islands and of the Seychelles cohorts	—	—	—	NOAEL = 1.2 μg Hg	6.4 [Intraspecies: 3.2, lack of data: 2]	—	0.19 Hg

Table 2. (*Continued.*)

Substance	Reference	Effect	Epidemiological study	Experimental study: Species	Experimental study: Exposure period	Experimental study: Exposure duration	Reference point (μg/kg/d)	Uncertainty factor	TT (μg/kg/d)	HBGV (μg/kg/d)
Polybrominated biphenyls (ΣPBBs)	Henck and Rech 1986	Dams were exposed to PBB (0.2 or 2 mg/kg/d) through gestation and lactation. PBB-exposed male and female offspring, subjected at 6 months of age to an autoshaping paradigm, exhibited differences in acquisition and performance in comparison with controls.	—	Sprague-Dawley rats	GD6–PND24	—	LOAEL = 200	300 [interspecies: 10, intraspecies: 10, LOAEL → NOAEL: 3]	0.7	—
Polybrominated diphenyl ethers (ΣPBDEs)	ATSDR 2017	Dams were exposed to PBDE-99 at 60 or 300 μg/kg/d. Exposure increased locomotor activity in offspring on PND36 and PND71 since the dose of 60 μg/kg/d (Kuriyama et al. 2005).	—	Wistar rats	GD6	Single dose	LOAEL = 60	1,000 [Interspecies: 10, Intraspecies: 10, LOAEL → NOAEL: 10]	—	MRL acute = 0.06
Polybrominated diphenyl ether 209 (PBDE 209)	ATSDR 2017	PND3 mice were exposed to PBDE-209 at 1.34, 2.22, 13.4, and 20.1 mg/kg BW/d. Exposed male mice showed altered spontaneous activity and reduced or lack of habituation at 2 and 4 months of age, in a dose-dependent manner. (Johansson et al. 2008)	—	NMRI mice	PND3	Single dose	NOAEL = 1340	100 [interspecies: 10, intraspecies: 10]	—	MRL acute = 10
Indicator polychlorinated biphenyls (ΣPCBi)	WHO 2003; ANSES 2007	In children: hypotonia and hyporeflexia at birth, delay in psychomotor development at 6 and 12 months, and poorer visual recognition memory at 7 months (Tilson et al. 1990).	Infants via breastfeeding	—	—	—	NOAEL = 0.093	12 [intraspecies: 3, extra factor: 2, ΣPCB → ΣPCBi: 2]	—	TDI = 0.01
		In male monkeys: exposure effects on a multiple fixed interval-fixed ratio schedule of reinforcement at 4 years of age, retarded learning, and perseverative behavior on a spatial delayed alternation task from 2.5 to 5 years of age (Rice and Hayward 1997, 1999).	—	Monkey (*Macaca fascicularis*)	Birth to 20 wk old	Repeated doses of a PCB mixture found in human milk	LOAEL = 7.5	600 [interspecies: 10, intraspecies: 10, LOAEL → NOAEL: 3], ΣPCB → ΣPCBi: 2	—	—
Dioxins and furans (ΣPCDD/Fs)	Zhang et al. 2018	Dams were exposed to TCDD at 0.2 or 0.8 μg/kg/d. TCDD at the lower dose induced premature sensory-motor development in preweaned male offspring. The higher dose induced, in addition, advanced eye opening prior to weaning and impaired spatial memory in adult males.	—	*Macaca fascicularis*	GD8–GD14	Repeated doses 6 d	LOAEL = 0.200	300 [interspecies: 10, intraspecies: 10, LOAEL → NOAEL: 3]	0.0007	—
Perfluorohexanesulfonic acid (PFHxS)	Viberg et al. 2013	PND10 mice were exposed to PFHxS at 0.61, 6.1, or 9.2 mg/kg. Exposed mice (2- and 4-month-old.) of both sexes exhibited altered adult spontaneous behavior and disrupted habituation in a novel home environment.	—	NMRI mice	PND10	Single dose	NOAEL = 6100	1,000 [interspecies: 10, intraspecies: 10, lack of data: 10]	6.1	—
Perfluorooctanoic acid (PFOA)	Goulding et al. 2017	Dams were exposed to PFOA at 0.1, 0.3, or 1 mg/kg/d. Exposed males displayed changes in locomotor activity on PND18–20, and dopamine-dependent motor behavior during adulthood for the higher dose.	—	CD1 mice	GD1–17	Repeated doses	NOAEL = 300	1,000 [interspecies: 10, intraspecies: 10, lack of data: 10]	0.3	—
Perfluorooctanesulfonic acid (PFOS)	Butenhoff et al. 2009	Dams were exposed to PFOS at 0.1, 0.3, or 1 mg/kg/d. Male offspring exposed to the higher dose displayed increased motor activity and reduced habituation on PND 17 but not on PND 13, PND 21 and PND 61.	—	Sprague-Dawley rats	GD0–PND20	Repeated doses	HED = 0.84	30 [interspecies: 3, intraspecies: 10]	0.03	—

Note: —, no data available; BW, body weight; GD, gestational day; HBGV, health-based guidance values; HED, human equivalent dose; ICR, inherited cataract rat; LOAEL, lowest observed adverse effect level; MRL, minimal risk level; NOAEL, no observed adverse effect level; PCB, polychlorinated biphenyl; PND, postnatal day; RfD, reference dose; TDI, tolerable daily intake; TT, toxicological thresholds.

Table 3. Exposures to the three selected mixtures by infant cluster and comparison with the exposure of the whole infant population.

			Cluster A: 55 infants				Cluster B: 91 infants				Cluster C: 34 infants			
				Exposure (μg/kg BW/d)				Exposure (μg/kg BW/d)				Exposure (μg/kg BW/d)		
Mixtures	Substances	Substance weight (%)	Mixture weight (%)	Mean	P95	Compared to all	Mixture weight (%)	Mean	P95	Compared to all	Mixture weight (%)	Mean	P95	Compared to all
Mixture 1	Hexachlorobenzene (HCB)	14.8	83.9	0.02	0.03	*** (<)	93	0.04	0.06	** (>)	47.5	0.04	0.06	—
	Dioxins and furans (ΣPCDD/Fs)	14.7	—	0.00001	0.00001	*** (<)	—	0.00002	0.00003	** (>)	—	0.00002	0.00003	* (>)
	ΣHeptachlor	11.7	—	0.01	0.01	*** (<)	—	0.02	0.03	*** (>)	—	0.02	0.03	—
	Indicator polychlorinated biphenyls (ΣPCBi)	10.3	—	0.12	0.28	*** (<)	—	0.31	0.56	** (>)	—	0.31	0.50	—
	ΣAldrine-dieldrin	8.0	—	0.01	0.02	*** (<)	—	0.03	0.05	** (>)	—	0.02	0.04	—
	Perfluorooctanesulfonic acid (PFOS)	7.3	—	0.003	0.01	*** (<)	—	0.005	0.01	—	—	0.01	0.02	*** (>)
	ΣChlordane-nonachlor	6.5	—	0.01	0.03	*** (<)	—	0.04	0.06	—	—	0.03	0.06	—
	Perfluorooctanoic acid (PFOA)	4.6	—	0.003	0.01	*** (<)	—	0.004	0.01	* (<)	—	0.01	0.02	*** (>)
	Pentachlorobenzene (PeCB)	4.4	—	0.001	0.003	—	—	0.002	0.004	—	—	0.002	0.004	—
	Polybrominated diphenyl ethers (ΣPBDEs)	3.7	—	0.003	0.01	*** (<)	—	0.01	0.01	—	—	0.01	0.02	—
	Perfluorohexanesulfonic acid (PFHxS)	3.0	—	0.001	0.002	—	—	0.001	0.002	—	—	0.002	0.005	*** (>)
	Polybrominated biphenyls (ΣPBBs)	2.7	—	0.0002	0.001	** (<)	—	0.0004	0.001	—	—	0.001	0.002	—
	Polybrominated diphenyl ether 209 (PBDE 209)	2.4	—	0.001	0.004	—	—	0.002	0.01	—	—	0.002	0.004	—
	ΣDDT/D/E	2.0	—	0.33	1.33	—	—	0.32	0.81	—	—	0.50	0.88	—
	Mirex	1.4	—	0.0003	0.001	** (<)	—	0.001	0.002	—	—	0.001	0.001	—
	Hexachlorocyclohexanes (ΣHCHs)	0.9	—	0.06	0.13	—	—	0.06	0.13	—	—	0.17	0.24	—
	Arsenic (As)	0.8	—	0.08	0.15	—	—	0.10	0.38	—	—	0.17	0.42	* (>)
	Inorganic mercury (inorganic Hg)	0.7	—	0.12	0.11	—	—	0.06	0.12	—	—	0.07	0.17	—
	Methylmercury (MeHg)	0.1	—	0.01	0.03	—	—	0.01	0.03	—	—	0.01	0.04	—
Mixture 2	ΣDDT/E/D	35.3	15.5	0.33	1.33	—	5.6	0.32	0.81	—	4.8	0.50	0.88	—
	Hexachlorocyclohexanes (ΣHCHs)	35.3	—	0.06	0.13	—	—	0.06	0.13	—	—	0.17	0.24	—
	Perfluorooctanesulfonic acid (PFOS)	15.8	—	0.003	0.01	*** (<)	—	0.005	0.01	—	—	0.01	0.02	*** (>)
	Polybrominated diphenyl ether 209 (PBDE 209)	5.2	—	0.001	0.004	—	—	0.002	0.01	—	—	0.002	0.004	—
	Indicator polychlorinated biphenyls (ΣPCBi)	3.5	—	0.12	0.28	*** (<)	—	0.31	0.56	** (>)	—	0.31	0.50	—
	Hexachlorobenzene (HCB)	2.0	—	0.02	0.03	*** (<)	—	0.04	0.06	** (>)	—	0.04	0.06	—
	Dioxin and furan sum (ΣPCDD/Fs)	1.9	—	0.00001	0.00001	*** (<)	—	0.00002	0.00003	** (>)	—	0.00002	0.00003	* (>)
	Pentachlorobenzene (PeCB)	0.7	—	0.001	0.003	—	—	0.002	0.004	—	—	0.002	0.004	—
	Mirex	0.2	—	0.0003	0.001	** (<)	—	0.001	0.002	—	—	0.001	0.001	—
Mixture 3	Perfluorononanoic acid (PFNA)	32.8	<1	—	—	—	1.8	0.0040	0.008	** (<)	47.6	0.004	0.025	*** (>)
	Perfluorooctanoic acid (PFOA)	29.9	—	—	—	—	—	0.001	0.00	* (<)		0.02	0.00	*** (>)
	Perfluorohexanesulfonic acid (PFHx)	16.3	—	—	—	—	—	0.005	0.009	—	—	0.004	0.016	*** (>)
	Perfluorooctanesulfonic acid (PFOS)	12.6	—	—	—	—	—	0.101	0.38	—	—	0.01	0.42	*** (>)
	Arsenic (As)	7.5	—	—	—	—	—	0.01	0.02	—	—	0.3	0.04	* (>)
	gamma-Hexachlorocyclohexane (gamma-HCH)	0.8	—	—	—	—	—	0.31	0.56	—	—	0.03	0.50	—

Note: "<" or ">" indicates a significant lower or higher mean exposure of the cluster compared to the mean exposure of the whole population using the 2-sided Student *t*-test. The mixture weight represents the participation of each mixture to the overall exposure of one cluster. For example, the exposure of cluster A was composed at 83.9% of mixture 1, 15.5% of mixture 2, and less than 1% of mixture 3. The substance weight represents the participation of each substance to the overall exposure of one mixture. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. —, no data available; BW, body weight; ΣDDT/E/D, Dichlorodiphenyltrichloroethane/dichlorodiphenyldichloroethylene/dichlorodiphenyldichloroethane.

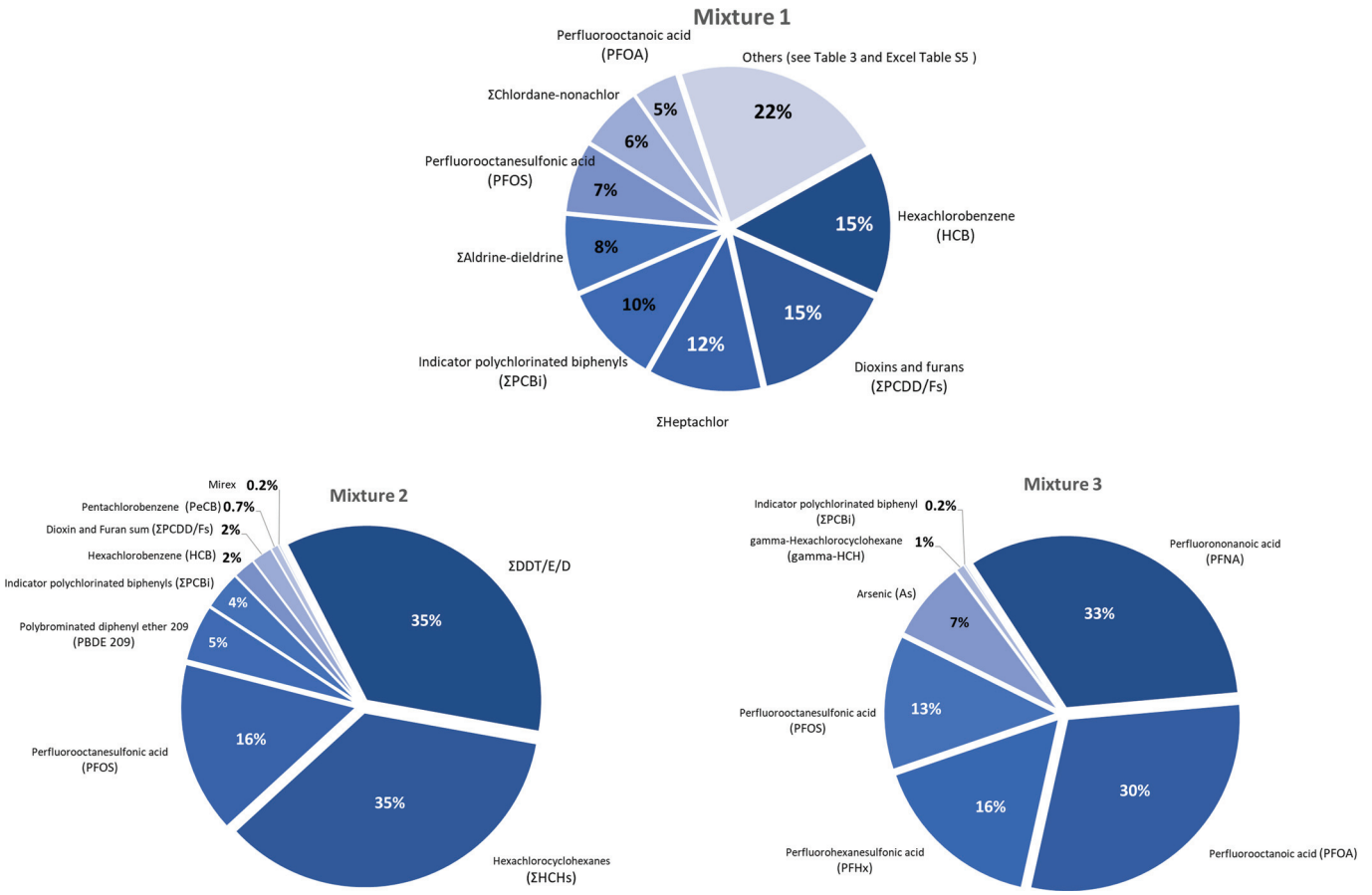


Figure 2. Detailed composition of the first three mixtures (pie charts). The percentage values in the pie charts are the statistical weights obtained in the W matrix for each substance representing their participation to each mixture (Excel Tables S2, S3, S4, and S5).

For thyroid effects, 13 among the 19 substances of mixture 1 were reported to have effects on this system (Table 1). HBGVs and TTs established on the basis of studies addressing gestational and/or lactational exposures have been defined for seven substances (ΣDDT, methyl mercury, ΣPBB, ∑PBDE, ΣPCBi, ∑PCDD/F and PFOS). HBGVs supported by epidemiological studies were available only for methylmercury and ∑PCDD/F. HBGVs based on animal experimental studies were reported for mirex and ΣPBDE. A TT was constructed for arsenic, ∑chlordane-nonachlor, ΣDDT, HCB, ΣPBB, PBDE-209, ΣPCBi, PeCB, and PFOS.

For neurodevelopmental effects, 17 substances were documented, with most of them taking into account infant sensitivity, except for ∑aldrin−dieldrin, which were tested in adult animals (Table 2). HBGVs based on neurodevelopmental effects were selected for six substances, i.e., ∑DDT, ∑Heptachlor, methylmercury, ∑PBDE, PBDE-209, and ∑PCBi. A TT for neurodevelopmental effects was built for the following substances: ∑aldrin-dieldrin, arsenic, ∑chlordane-nonachlor, HCB, ∑HCH, inorganic mercury, ∑PBB, ∑PCDD/F, PFHxS, PFOA, and PFOS.

Table 4 shows that among the 19 analyzed substances, 8 substances had an HBGV or TT for only one end point (thyroid effect: mirex and PeCB; neurodevelopmental effect: ∑aldrin-dieldrin, ΣHCH, Σheptachlor, inorganic mercury, PFHxS and PFOA). Because the two studied effects were biologically linked, when a TT or the HBGV was missing for one end point, the value available for the other end point was used. The other 11 substances had a retained value for both thyroid and neurodevelopmental effects. Comparison of these values between the two end points showed that they were identical for methylmercury and ∑PBDE. The values were derived, respectively, from the same human and animal studies assessing the two end points, indicating similar sensitivity of the two systems for these substances. The values presented a slight difference with a neurodevelopmental/thyroid ratio around 0.6 for ∑PBB, PBDE-209, and PFOS, also suggesting comparable sensitivity of the thyroid and nervous system in particular for PFOS, which was tested for the two end points using comparable doses. The values of the other substances presented a neurodevelopmental:thyroid ratio that was either low at 0.2 for ∑PCBi and 0.08 for ∑chlordane-nonachlor, or high in the range of 12 for HCB, 40 for arsenic and ∑DDT, and 1,000 for ∑PCDD/F.

Risk of thyroid and neurodevelopmental effects. All individual mRPIs of mixture 1 were higher than 1 for both thyroid and neurodevelopmental effects (Figure 3; Excel Tables S8 and S9). The values of mRPI obtained for the thyroid effect were overall three times higher than those obtained for the neurodevelopmental effect. For each effect, four main substances contributed to the risk. ∑DDT, ∑PCBi, and arsenic contributed to the risk for both effects in different proportions.

For the thyroid effect, the contributions of total population were balanced among ∑PCDD/F, ∑DDT, and arsenic (Figure 4). The contribution of ∑PCBi was much lower (around 6%). The contributions of cluster 2 were similar to that of the total population, whereas the contributions of ∑PCDD/F for cluster 1 and of arsenic for cluster 3 were slightly higher.

In contrast, for the neurodevelopmental effect, the risk was driven by ∑PCBi for the total population (88%) and the three clusters (83%, 89%, and 91%) (Figure 5). ∑DDT, arsenic, and ∑aldrin-dieldrin contributed in similar proportions (<3.2%), except for cluster 1, where ∑DDT had a higher contribution (6.1%).

Table 4. HBGV and TT values (micrograms per kilogram per day) for the thyroid and the neurodevelopmental effects and ratio between both effects.

Substances	Thyroid effect		Neurodevelopmental effect		Neurodevelopmental/thyroid ratio
	Retained value	Reference	Retained value	Reference	
ΣAldrin-dieldrin	—	—	0.03	Smith et al. 1976	—
Arsenic (As)	0.005	Sun et al. 2017	0.2	Tsuji et al. 2015	40
ΣChlordane-nonachlor	4.2	Bondy et al. 2004	0.33	Cassidy et al. 1994	0.1
Dichlorodiphenyl-trichloroethanes/dichloroethylene/dichloroethane (ΣDDT/D/E)	0.012	Yaglova et al. 2016, 2018	0.5*	ATSDR 2002	42
Dioxins and furans (ΣPCDD/Fs)	0.0000007*	U.S. EPA 2012	0.0007	Zhang et al. 2018	1000
ΣHeptachlor	—	—	0.1*	ATSDR 2007	—
Hexachlorobenzene (HCB)	0.53	Hadjab et al. 2004	6.4	Lilienthal et al. 1996	12
Hexachlorocyclohexanes (ΣHCHs)	—	—	0.62	Johri et al. 2007	—
Indicator polychlorinated biphenyls (ΣPCBi)	0.05	Provost et al. 1999	0.01*	WHO 2003, ANSES 2007	0.2
Inorganic mercury (inorganic Hg)	—	—	1.23	Huang et al. 2011	—
Methylmercury (MeHg)	0.19*	EFSA 2012	0.19*	EFSA 2012	1
Mirex	0.2*	U.S. EPA 1992	—	—	—
Pentachlorobenzene (PeCB)	2.7	McDonald 1991	—	—	—
Perfluorohexanesulfonic acid (PFHxS)	—	—	6.1	Viberg et al. 2013	—
Perfluorooctanesulfonic acid (PFOS)	0.05	Lau et al. 2003	0.03	Butenhoff et al. 2009	0.6
Perfluorooctanoic acid (PFOA)	—	—	0.3	Goulding et al. 2017	—
Polybrominated biphenyls (ΣPBBs)	1.25	Werner and Sleight 1981	0.7	Henck and Rech 1986	0.6
Polybrominated diphenyl ethers (ΣPBDEs)	0.06*	ATSDR 2017	0.06*	ATSDR 2017	1
Polybrominated diphenyl ether 209 (PBDE 209)	16.7	Wang et al. 2019	10*	ATSDR 2017	0.6

Note: * indicates HBGVs. —, no data available; HBGV, health-based guidance values; TT, toxicological thresholds.

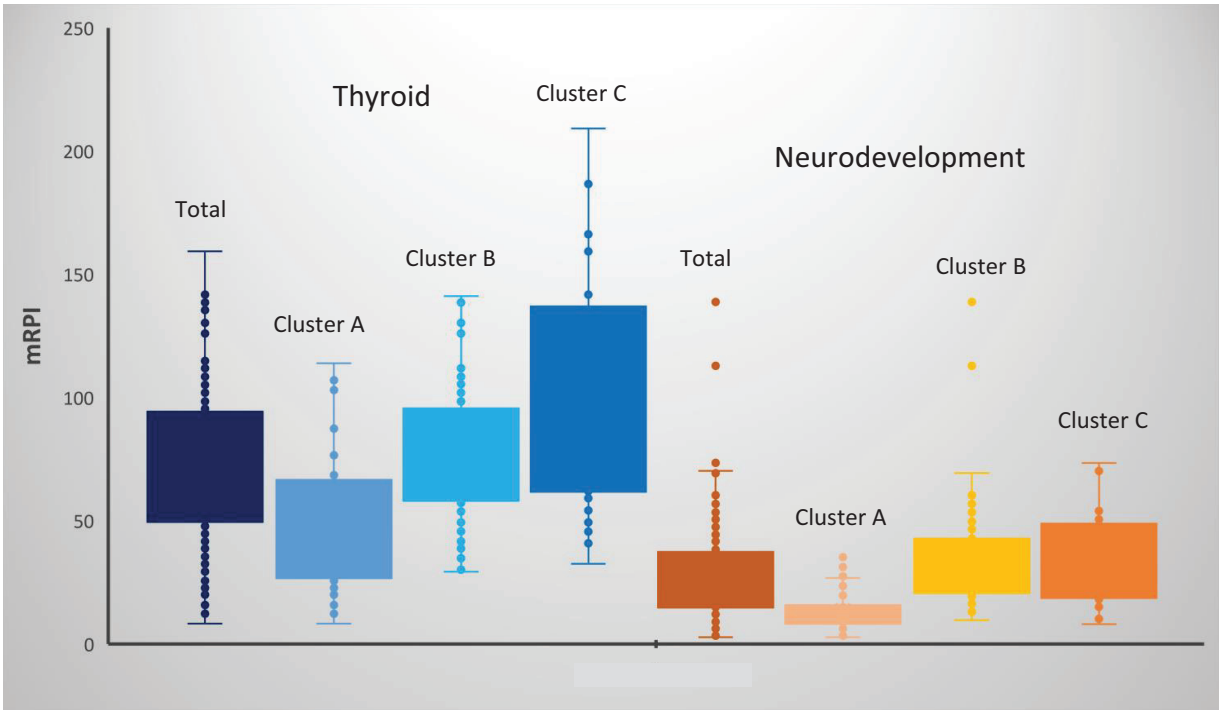


Figure 3. Box plots of the modified reference point index (mRPI) distribution for the total population and for the three population clusters for thyroid and neurodevelopmental effects (Excel Tables S6 and S7). The box lines represent the first (Q1) and third (Q3) quartiles, and the middle line the second quartile (Q2). The whiskers were calculated by min [all values, number of values $\leq Q3 + (Q3 - Q1) \times 1.5$] and max [all values, number of values $\geq Q1 + (Q3 - Q1) \times 1.5$]. The outlier points defined as values higher than the upper or lower whiskers were not drawn for the thyroid effect for graphical readability. Note: min, minimum; max, maximum.

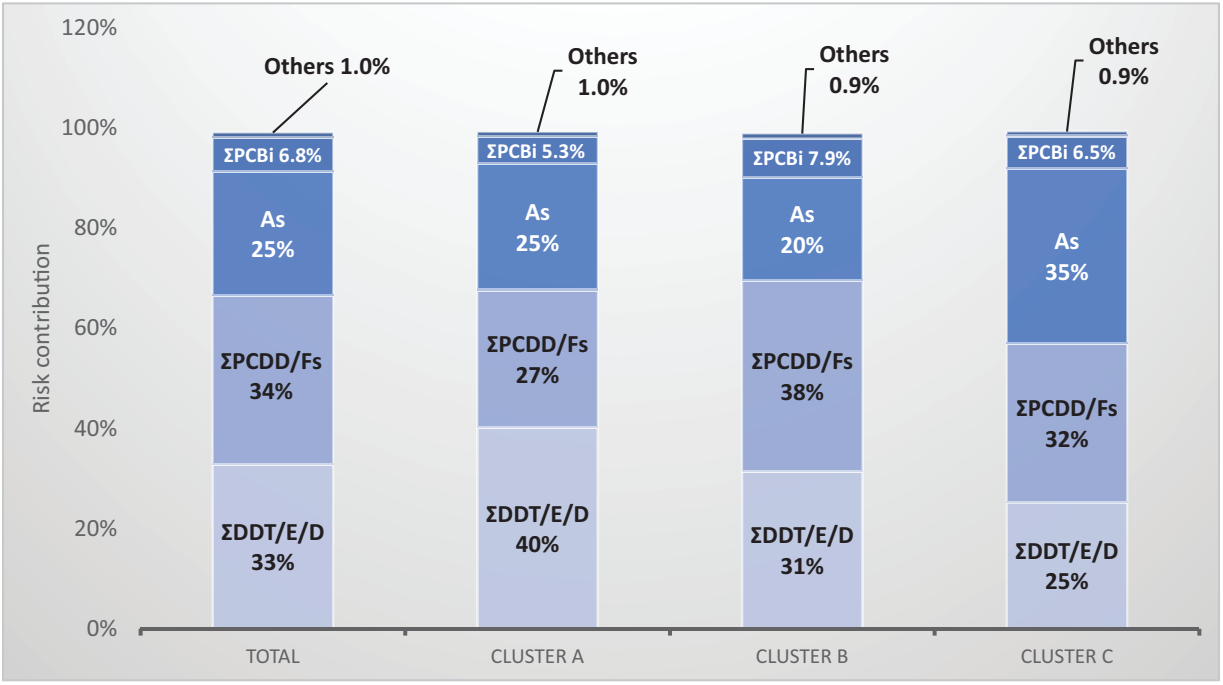


Figure 4. Contributions to the risk of thyroid effect for the four main contributing substances.

Discussion

The present work proposes an integrated approach for risk assessment of combined exposure to contaminant mixtures. This method is based on the latest methods developed in the field of chemical mixtures to select mixture components from combined exposure and to estimate the associated cumulative risk. We started from observed coexposures to select substances mainly present together and for which potential mixture effects must be studied as a priority. After selecting common relevant effect(s) for the studied population, we proposed to use available HBGVs or to construct TTs considering specific end points to estimate the cumulative risk related to the selected mixtures.

Our approach deals with substances belonging to different chemical classes and different regulatory silos, unlike previous methods based on TEFs [e.g., dioxin TEQs (Van den Berg et al. 2006)] focusing on one chemical class, or based on cumulative assessment groups developed for pesticide regulation (EFSA 2013c; EFSA Scientific Committee 2019). Importantly, in the context of *a posteriori* risk assessment, mixtures are driven by substances found in the environment and must be studied in a way that corresponds to the reality of current exposure (Kortenkamp and Faust 2018; Sprong et al. 2020). However, due to the high number of substances present in the environment [for example more than 400 analyzed in food in the French TDS2 (Sirot et al. 2009)], the number of mixture possibilities is infinite, and studying the entire

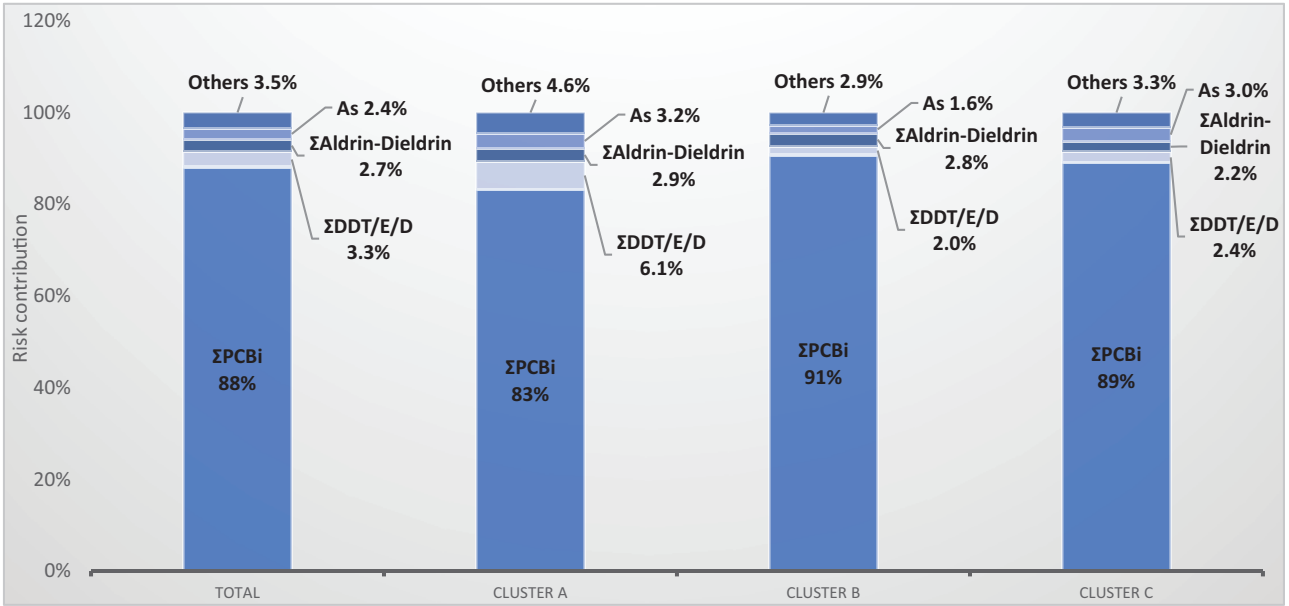


Figure 5. Contributions to the risk of neurodevelopmental effect for the four main contributing substances.

mixture, i.e., composed of all substances present in a media, is unrealistic in most cases. As a result, there is a need to prioritize the mixture components that will be studied for risk assessment. Our approach proposes to define relevant mixtures by studying exposure correlations and thus to select substances with the highest probability of being found together in a given time frame. In the present case study on human breast milk contamination, this approach makes it possible to highlight certain substances that would not have been considered in the first instance for the selected effects; such is the case for arsenic, which is regulated on the basis of its effects on the skin, lungs, and the urinary bladder (EFSA 2009). Some of the co-occurring substances may present scarce toxicological data regarding the selected effects, therefore possibly introducing substantial uncertainty. This method also implies that some substances (such as those with low exposure levels, those not often found in combination with other substances, or those that concern a small number of people in the population) are not considered in the mixture. Even though some of these substances may have high toxicity, due to their low exposure their contribution to the risk is considered to be weak. For example, in our case study, lead, a well-known neurodevelopmental toxicant (Lidsky and Schneider 2003), was not included to the selected mixture because of very low concentrations in breast milk with high censored data. In our example, because the mRPI values were already very high and lead exposure very low, adding lead or not in the mixture would not have changed the risk conclusion. However, in other cases with mRPI values closely lower to 1, it could have made a difference. Thus, it is important to go back to the substances not selected and discuss for the major ones their potential impact on risk conclusions. The mixture 3 was also not studied because it concerned a small number of people and thus explained a little part of the variance. However, in future work, it would be interesting to study this mixture specifically composed of perfluorinated compounds to investigate the mothers' diets to find the main contributors and their link to specific diets like vegetarian or to other lifestyle factors. Another point is that the number of studied substances is limited by the analytical design of the survey and the associated LR. With the LB option, substances that were not measured in the survey, or for which all values were below the LR, were not included in the mixture. The impact of the censored options on the mixture components and on the risk assessment conclusions can be tested by comparing results with MB and UB. In our case study because mRPI were already higher than 1, we considered it not necessary to go further. Finally, selecting mixture components from combined exposure may result in considering substances with poor toxicological data regarding the selected effects and thus with high uncertainty on the constructed TT. This is the case with arsenic, PFHsX, and PFOA, for which an additional UF of 10 was applied to account for lack of data regarding neurodevelopmental end points selected in the present study.

We proposed selection of specific end points, taking into account two criteria: the most common reported effect or effects for the mixture components and their relevance regarding the target population. If the target population were adults exposed by food, hepatotoxicity would certainly have been selected because it is one of the most common effects reported in publications for the selected substances. In the context of the general population and with various observed effects, the choice is less obvious. In this case, several effects can be studied, depending on the time and means allocated to risk assessment. In our case study focusing on infants, it was relevant to select neurodevelopment and thyroid effects. We chose to complete the analysis of neurodevelopmental effects using data on thyroid effects because changes in thyroid hormone levels can lead to abnormal brain development, as previously mentioned and reported in humans and experimental models (Prezioso et al. 2018; WHO 2012). To strengthen the link between the two chosen effects, we focused on neural functions and behaviors largely known to be regulated by thyroid hormones, such as cognitive and sensorimotor/motor processes. In the same way, for the thyroid effects, we focused on changes in hormonal levels known to directly affect neurodevelopment, with a particular focus on changes in thyroxin levels. Some HBGVs (for $\sum$PBDE and methylmercury) were in fact based on similar neural and thyroid end points, which were analyzed in the same studies. An interesting observation was that several substances present in studied mixture 1 had already been listed as compounds with recognized thyroid-disrupting activities (Demeneix 2019; Mughal et al. 2018; WHO 2012). This observation was the case with some legacy organohalogen compounds (DDT, HCB, PCB, PCDD/F, and PBDE) and perfluorinated chemicals including PFOS, PFOA, and PFHxS. The addition of remaining substances for which identified studies also reported thyroid effects gave a total of 13 substances with thyroid effects among the 19 substances in mixture 1. Overall, these observations led us to suggest that about 70% of substances in mixture 1 exhibit a joint mode of action, i.e., disruption of the hormonal thyroid system, which can then underlie the adverse neural effects, in particular for developmental exposure. In this context, the additivity hypothesis was considered the most relevant.

In our case study, when defining TTs for the two selected end points, the values of some substances ($\sum$aldrin-dieldrin, arsenic, $\sum$chlordane-nonachlor, and $\sum$DDT) were lower than the regulatory HBGVs based on other effects. Therefore, in these particular cases, the HI would not have been such a conservative scenario. The HI or the RPI has been proposed to calculate cumulative risks (EFSA 2019). However, because they are based on the most sensitive end point, which can be different from one substance to another (HI) or use a common generic UF (RPI), they were found to be rather conservative and can only be used in a tier 0 approach. Applying such methods seems of no use when studying contaminants for which exposure levels are often much higher than the HBGVs and thus will lead to refinement in considering joint specific effects. The mRPI proposed by Vejdovszky et al. (2019) was well adapted to our case study to characterize the risk. Clearly the mRPI was especially developed to consider specific effects and to integrate heterogeneous UFs. However, our approach to establish UF of TT was different from that of Vejdovszky et al. (2019), who considered that a specifically derived UF for any adverse effect should not be more conservative than the HBGV. In the case of a UF leading to a toxicological value lower than the HBGV, they proposed that the mRPI calculations should simply use the HBGV instead of the TT for this substance. For our case study, we proposed lower TTs because *a*) the HBGV was built from adult experimental data and needed to be adapted to infants, such as data for $\sum$aldrin-dieldrin and chlordane (for these two cases, the TTs were found to be very close to the HBGV); *b*) new data were available since the construction of the HBGV, such as epidemiological data highlighting neurodevelopmental effects of arsenic exposure in children; and *c*) the studied specific effect was not taken into account to construct the HBGV due to a lack of data, such as data for legacy organochlorine substances (chlordane, $\sum$aldrin-dieldrin) for which neurodevelopmental effects were not considered at that time. This earlier lack of data was also the case for arsenic for the thyroid effect.

Our methodological approach considering two end points was reinforced by the fact that risk results were consistent between the neurodevelopmental and the thyroid effects. We observed that $\sum$DDT, $\sum$PCBi, and arsenic mainly contributed to the risk of the two effects. Each substance or sum of substances had its own

HBGV or TT value for each effect; thus, similarities were not due to replacement of HBGV values between the two effects. The fact that ∑DDT and ∑PCBi participated in this trio was consistent with the evidence in the extensive literature reporting their thyroid and neural effects. The thyroid effects of ∑PCDD/F, derived from human data, were reinforced by environmental experiments because these substances were shown to drive the effects (reduced T4 levels) induced by prenatal and postnatal exposure of rats to an arctic mixture composed of 27 chemicals (Elabbas et al. 2014).

Comparison between the mRPI values for neurodevelopmental and thyroid effect showed that mRPI values of thyroid effects were overall three times higher than those obtained for the neurodevelopmental effect. This result should be interpreted with caution, bearing in mind the robustness of HBGV derivation. Although five substances presented either identical or very close HBGVs (methylmercury, ∑PBB, ∑PBDE, PBDE-209, and PFOS), suggesting similar sensitivity for the two end points, three substances (arsenic, ∑DDT, and HCB) had less robust thyroid TT values. Importantly, the TT values built for the three substances were derived from a few recent studies that investigated low doses, thereby resulting in lower TT for thyroid effects (Tables 2 and 3). The thyroid effects of these substances need to be investigated more closely in the future.

The consistency of the risk results between both end points supported the statement that the risk of mixtures regarding breast milk feeding was mainly driven by a small number of substances. Despite the fact that organochlorine compounds (PCDDs and PCDFs, PCBs, and DDT) have been regulated since the 1980s and are prohibited by the Stockholm Convention, their contamination levels in humans and the environment are still high (Komprda et al. 2019; Landrigan 2020; UNEP 2020).

Another important contribution of our approach was that considering mixtures highlights substances that would not have been considered in single risk assessments for the studied effects. This result was the case with arsenic and ∑aldrin-dieldrin, which appeared to be significant contributors to the risk of neurodevelopmental effect, whereas their chronic HBGVs were based on skin lesions for arsenic and hepatic effects for ∑aldrin-dieldrin (ATSDR 2020). To reduce the risk of mixture for infants, policy makers should consider these substances together to propose suitable management options. The results of the ContaLait survey should be confirmed by a more extensive survey of nursing mothers in France. It would also be relevant to investigate substances found in other breast milk surveys such as pyrethroids, chlorpyrifos, phthalates, bisphenols, etc. (Kim et al. 2018; Weldon et al. 2011). Moreover, breastfeeding is known to present several benefits, and the WHO recommends that mothers exclusively breastfeed infants for a child's first 6 months to achieve optimal growth, development, and health (van den Berg et al. 2017).

In conclusion, we propose a complete step-by-step method to assess the risk of contaminant mixtures. This method starts from combined exposure and progresses to prioritize substances and then apply a component-based approach to cumulate the risk under dose additivity. This method is of particular interest when risk assessment must be done across silos regarding an extensive range of substances present in an environmental matrix. This range of substances could be, as proposed here, contaminants in breast milk, but more generally contaminants in food or in water, air, soil, or consumer products. This approach can be applied to the different available data sets to yield exposure estimates through *a*) linking consumer behaviors (food consumption, usage of consumer products, time spent in activities and in locations and microenvironments) and contaminant concentrations in different environmental media; *b*) using body burdens from human biomonitoring surveys; or *c*) using any type of data that constitutes a proxy of exposure. The application of this approach to our case study demonstrates the importance of crossing silos when studying mixtures and of identifying risk drivers to regulate the risk related to environmental contamination. Applying this method to other types of data, for example on human biomonitoring to account for all sources of exposure, or in ecotoxicology, will reinforce its relevance for risk assessment. This work also highlighted the importance of improving the efficiency of mixture risk assessment and of continuing the efforts to collect and structure toxicological data. HBGVs are already part of national (www.anses.fr), European (OpenFoodTox data), and American (ATSDR) initiatives. These data sets provide information about critical effects but reveal nothing about other toxicological effects. There is an urgent need for mixtures assessments to also gather reference points on main toxicological issues and make them available to risk assessors.

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