Impact of mixtures of persistent organic pollutants on breast cancer aggressiveness

Louise Benoit, Meriem Koual, Céline Tomkiewicz, Anne-Sophie Bats, Jean-Philippe Antignac, Xavier Coumoul, Robert Barouki, Germán Cano-Sancho

Environment International 170 (2022) 107615

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

Impact of mixtures of persistent organic pollutants on breast cancer aggressiveness

Louise Benoit [a,b,c,*], Meriem Koual [a,b,c], Céline Tomkiewicz [a], Anne-Sophie Bats [b,c,e], Jean-Philippe Antignac [d], Xavier Coumoul [a,c], Robert Barouki [a,c], German Cano-Sancho [d]

[a] INSERM UMR-S 1124, T3S, Toxicologie Environnementale, Cibles thérapeutiques, Signalisation cellulaire et Biomarqueurs, France
[b] Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges-Pompidou, Service de Chirurgie Cancérologique Gynécologique et du Sein, Paris, France
[c] Université Paris Cité, 45 rue des Saints-Pères, 75006 Paris, France
[d] LABERCA, Oniris, INRAE, 44307 Nantes, France
[e] INSERM UMR-S 1147, Université Paris Cité, Centre de Recherche des Cordeliers, Paris, France

ARTICLE INFO

Handling editor: Marti Nadal

Keywords:
Adipose tissue
Mammary cancer
Endocrine disrupting chemicals
Environmental exposure
Metastasis

ABSTRACT

Introduction: Breast cancer (BC) is frequent with a poor prognosis in case of metastasis. The role of the environment has been poorly evaluated in its progression. We searched to assess whether a mixture of pollutants could be responsible of BC aggressiveness.

Methods: Patients undergoing surgery for their BC were prospectively included in the METAPOP cohort. Forty-two POPs were extracted, among them 17 dioxins (PCDD/F), 16 polychlorobiphenyls (PCB), 8 polybromodiphenylethers (PBDE) and 2,2′,4,4′,5,5′-hexabromobiphenyl (PBB153) were measured in the adipose tissue surrounding the tumor. BC aggressiveness was defined using tumor size and metastasis (distant or lymph nodes). Two complementary models were used to evaluate the impact of the mixture of pollutants: the BKMR (Bayesian Kernel machine regression) and WQS (weighted quantile sum regression) models. The WQS estimates the weight (positive or negative) of a certain chemical based on its quantile and the BKMR model applies a kernel-based approach to estimate posterior inclusion probabilities. The sub-group of patients with a body mass index (BMI) > 22 kg/ m^2 was also analyzed.

Results: Ninety-one patients were included. Of these, 38 patients presented a metastasis, and the mean tumor size was 25.4 mm. The mean BMI was 24.5 kg/m^2 (+/- 4.1). No statistical association was found in the general population. However, in patients with a BMI > 22 kg/ m^2, our mixture was positively associated with tumor size (OR: 9.73 95 %CI: 1.30–18.15) and metastasis (OR = 3.98 95 %CI = 1.09–17.53) using the WQS model. Moreover, using the BKMR model on chemical families, dioxin like chemicals and PCDD were associated with a higher risk of metastasis.

Discussion: These novel findings identified a mixture associated with breast cancer aggressiveness in patients with a BMI > 22 kg/ m^2.

1. Introduction

Breast cancer is the most frequent female malignancy and it was responsible for 684 996 deaths in 2020 worldwide (Globocan, 2012). The five-year survival rate is 90 % when the cancer is diagnosed at an early stage. This rate drops drastically to 86 % and 28 % when the disease spreads to the lymph nodes or is characterized by distant metastasis, respectively (Siegel et al., 2021). The incidence of breast cancer has been associated with age, genetic mutations (e.g. BRCA 1, 2), obesity or hormonal exposure (Sun et al., 2017), however, risk factors for breast cancer progression and metastatic evolution are poorly known and the molecular processes involved in the initiation/promotion of a cancer cell are completely different than the ones triggering metastasis.

There is a growing concern about the role of the environment on breast cancer progression, notably persistent organic pollutants (POPs) a vast family of environmental pollutants listed in the Stockholm Convention and strongly regulated worldwide due to its hydrophobic, persistent and toxicological properties (Koual et al., 2020). For instance,

* Corresponding author at: INSERM UMR-S 1124, T3S, Toxicologie Environnementale, Cibles thérapeutiques, Signalisation cellulaire et Biomarqueurs, France.
E-mail address: louise.am.benoit@gmail.com (L. Benoit).

https://doi.org/10.1016/j.envint.2022.107615
Received 27 July 2022; Received in revised form 27 September 2022; Accepted 2 November 2022
Available online 3 November 2022

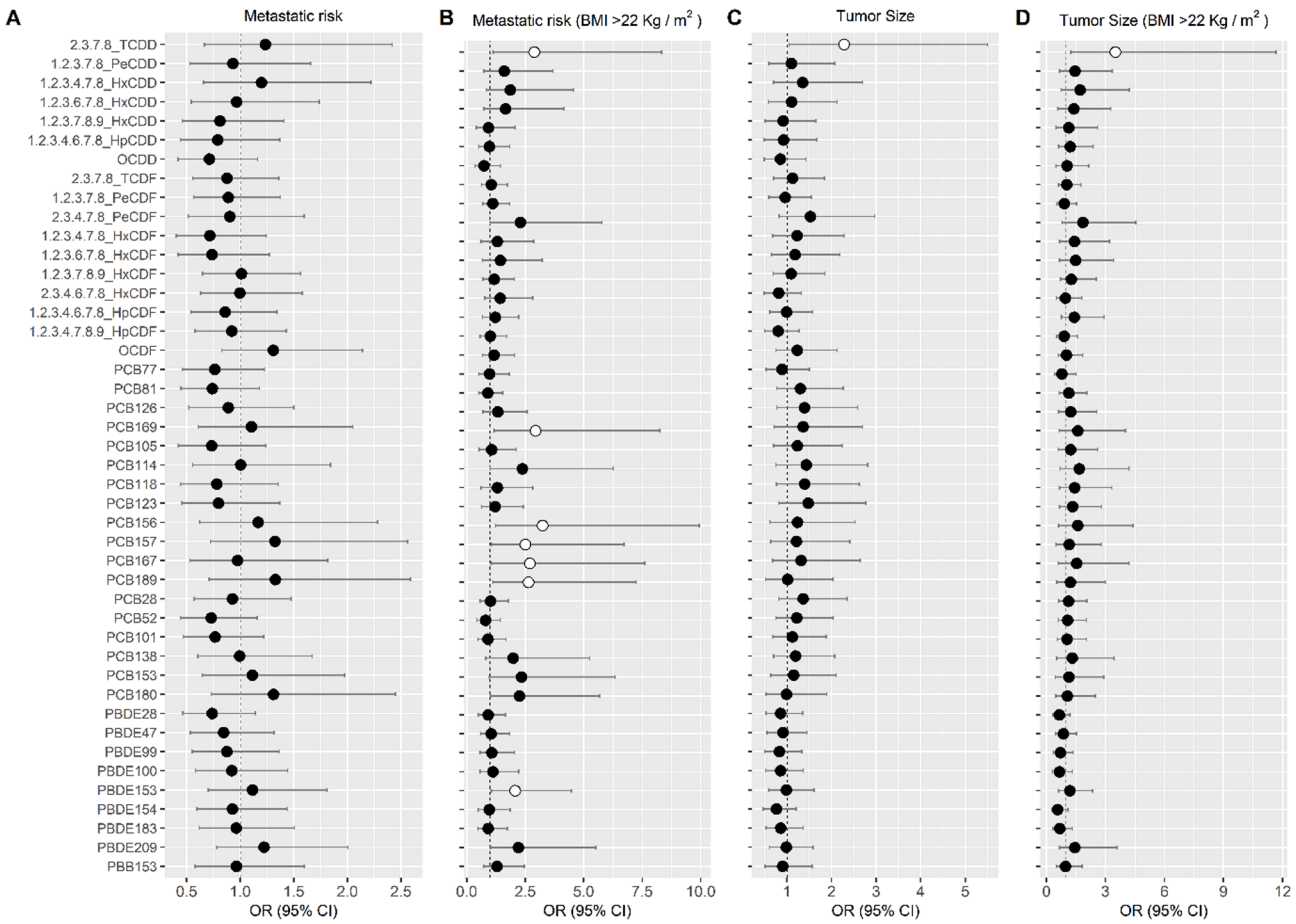

Fig. 1. Forest plots depicting the associations between persistent organic pollutants and metastatic cancer risk for all women (Panel A) or only women with body mass index above 22 kg/m^2 (Panel B), or tumor size (dichotomized at 20 mm, Panel C and D). Results are represented by the respective odds ratios (OR) and 95 % confidence intervals (CI). All models were adjusted by age, body mass index, personal history of breast cancer, smoking and menopause status. Statistically significant associations are highlighted with white circles.

we have previously found that low-doses of pollutants such as the 2.3.7.8-tetrachlorodibenzo-p-dioxin (TCDD) and coplanar polychlorinated biphenyls (PCB) 77 and 169 were individually associated with breast cancer metastasis and tumor size (Koual et al., 2019). Those associations were substantially strengthened among overweight women (body mass index, BMI $>$ 25 kg/m^2) suggesting an effect modification driven by adiposity as discussed elsewhere (Bokobza et al., 2021). Other studies also support the link between several POPs and tumor size or breast cancer recurrence, however, in all cases, each chemical was studied individually (Demers et al., 2000; Høyer et al., 2001; Muscat et al., 2003) yet complex mixtures of POPs are likely to occur.

Single pollutant models are constrained to recapitulate the real-life scenarios, hence, there is an increasing interest in developing and applying novel statistical approaches to assess the impact of pollutants as mixtures (Carlin et al., 2013; Lee et al., 2017). Indeed, people are exposed during the entire lifespan to multiple chemicals from different environmental sources, through different routes and duration of exposure, resulting in diversified and dynamic patterns of accumulation (Drakvik et al., 2020; Barouki et al., 2022). During the last years, chemical mixtures has been listed among public health priorities and have been addressed in regulations around the world (Drakvik et al., 2020; Barouki et al., 2022) to better represent the daily real-life in chemical risk assessment (Woodruff et al., 2011). The joint effect of chemical mixtures takes into account the cumulative effects and interactions between pollutants such as: additive (the effect of a mixture is the sum of pollutant A and B), synergistic (the effect is superior to the sum of A and B), potentializing (A alone has no effect, but has an effect only in the presence of B), antagonistic (the effect is inferior to the sum of A and B) or dose dependent (Braun et al., 2016). The joint effect can be measured using the maximum cumulative ratio or the summation of concentrations grouping pollutants by class such as structural similarity, biological class or toxic equivalent factors (TEF) (Burns et al., 2011; Price and Han, 2011).

During the last few years, different statistical methods have been used to assess the effect of a mixture in observational studies, with a particular interest on weighted quantile sum regression (WQS) and Bayesian Kernel machine regression (BKMR) (Gibson et al., 2019). On the one hand, WQS estimates the weight (positive or negative) of a certain chemical based on its quantile (Carrico et al., 2015). On the other hand, the BKMR model applies a kernel-based approach to estimate the exposure–response function incorporating complex interactions between pollutants, with flexibility to fit nonlinear associations (Bobb et al., 2015). Whereas WQS provides a straightforward and interpretable way to assess the overall effect of a mixture dealing with collinearity issues, the method may be constrained by the assumptions of directionality, presence of interactions or non-linearity (Lazarevic et al., 2019).

The general aim of our study was to extend our previous single-pollutant analysis (Koual et al., 2019) and to characterize the impact of mixtures of POPs in adipose tissue from women with breast cancer based on different hallmarks of aggressiveness using two complementary analyses: the BKMR and WQS models. Specifically, we aimed: a) to characterize the joint effect of chemicals, b) to identify relevant chemicals within the mixture and c) to gain insight about potential

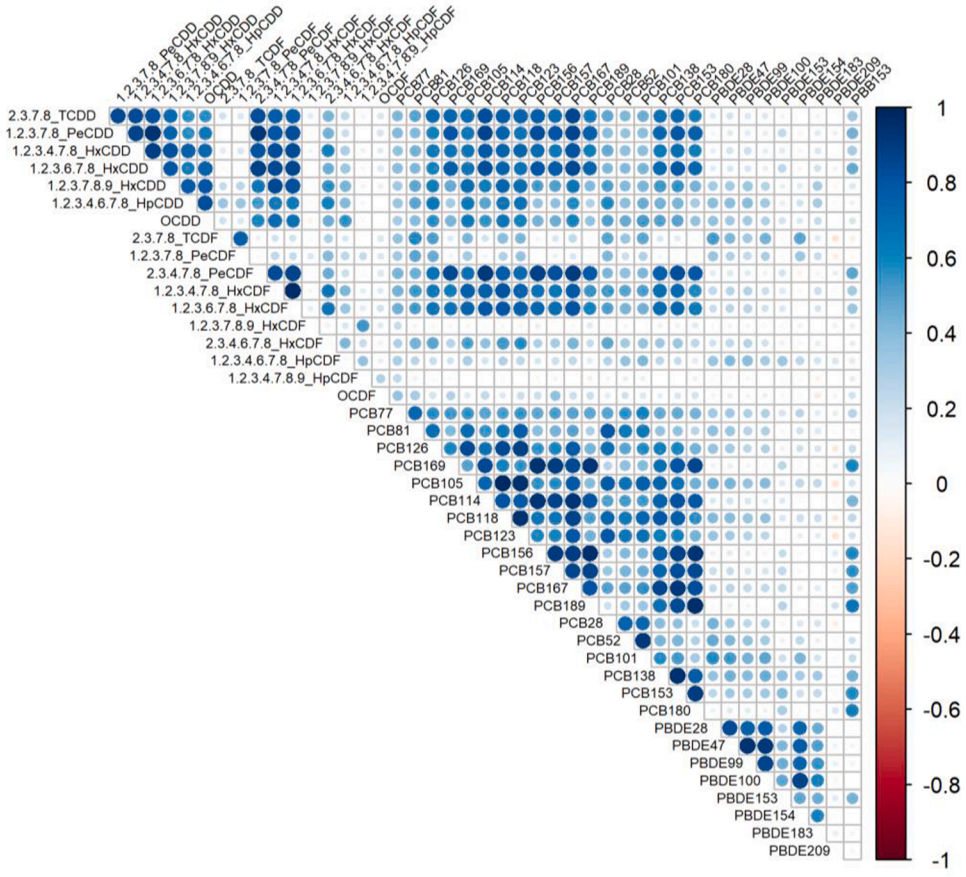

Fig. 2. Heatmap displaying the Spearman coefficients for the bivariate correlations between chemicals.

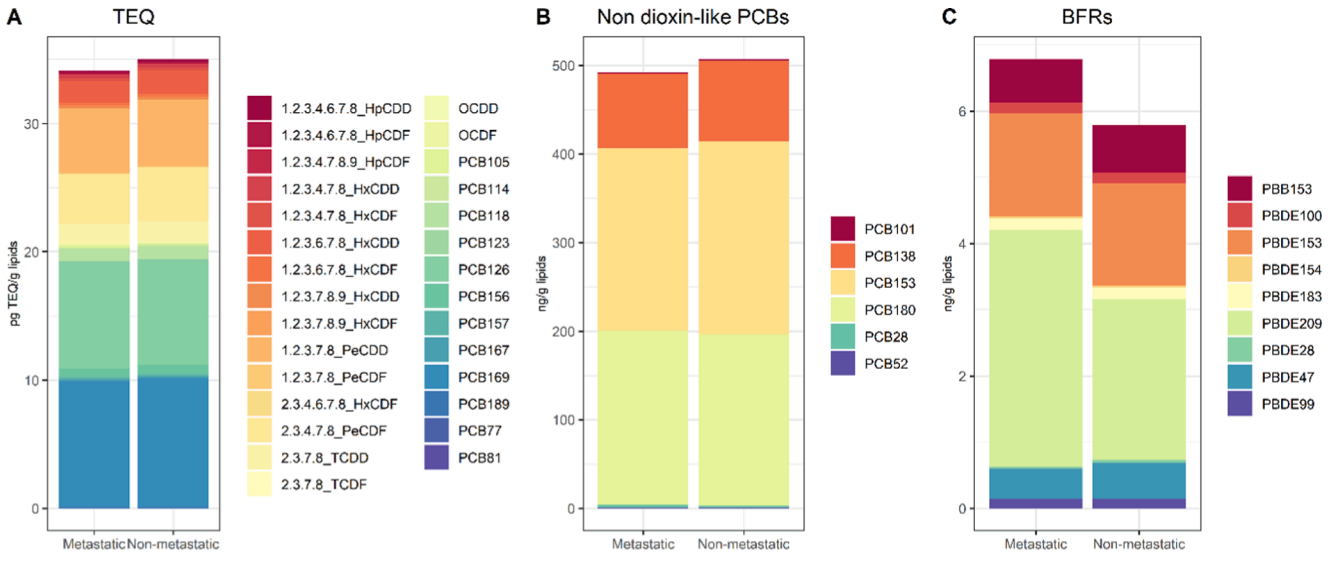

Fig. 3. Concentrations of chemicals in adipose tissue from patients with metastatic and non-metastatic breast cancer. Panel A depicts toxic equivalents (TEQ) for dioxin-like chemicals, panel B depicts the concentrations of non-dioxin like polychlorinated biphenyls (PCBs) and panel C brominated flame retardants (BFRs).

interactions between chemicals.

2. Material and methods

2.1. Study population

The present study uses data from METAPOP study described elsewhere (Koual et al., 2019). This monocentric cohort comprised of patients with breast cancer followed at the department of gynecological-oncological surgery at the Georges-Pompidou European Hospital (HEGP, Paris, France) from December 2013 until November 2017. Included participants were female patients over 18 years old, presenting a newly diagnosed breast cancer and benefitting from surgery for a uni- or multifocal lesion with the main lesion being > 1 cm in size or

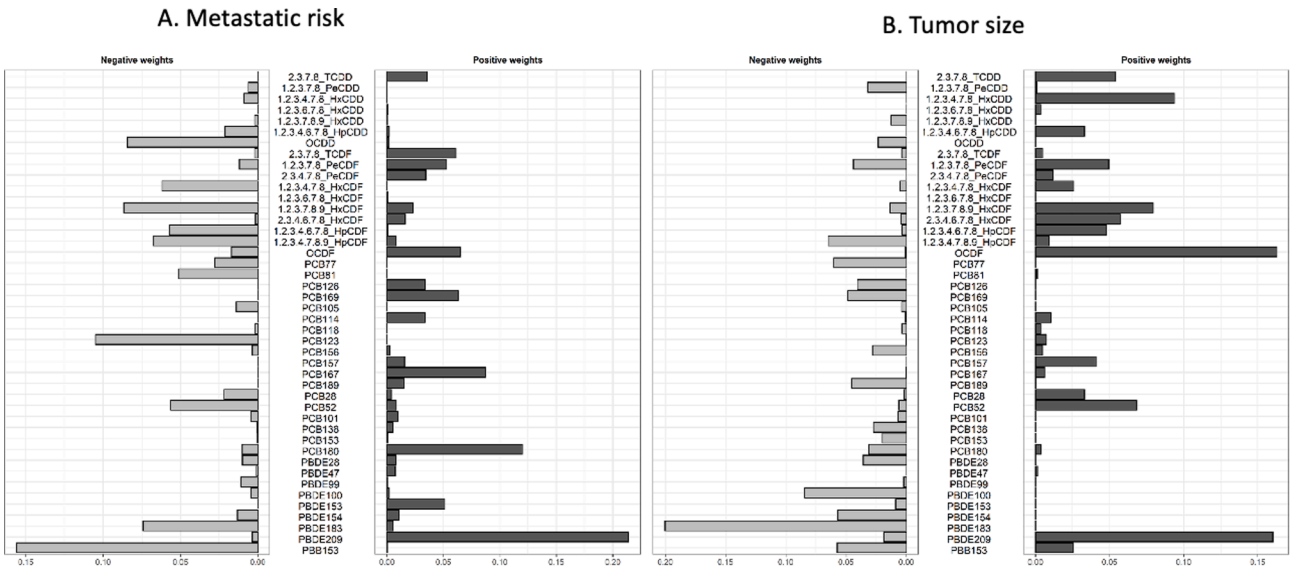

Fig. 4. Identification of relevant chemicals in the mixture using weighted quantile sum regression (WQS). Bar plot depicting the negative and positive weights attributed to each persistent organic pollutant in the mixture model for metastatic risk (Panel A) or tumor size (Panel B) using weighted quantile sum regression, for the sub-group of participants with body mass index above 22 kg/m$^{2.}$

palpable. Patients with a non-palpable lesion, male patients or patients already participating in multiple study protocols were excluded. All patients gave informed consent to participate in the study. The study was approved by the 'Comité de Protection des Personnes' in 2013 [French equivalent of an Institutional Review Board (IRB)].

2.2. Data collection and outcome ascertainment

Patients benefitted from a partial or total mastectomy. Lymph node biopsy and/or axillary lymph node removal (depending upon the lymph node biopsy results) were performed according to the American Society of Clinical Oncology (ASCO) guidelines. The malignant tissue removed during the surgery was sent for pathology assessment. During surgery, an adipose tissue sample (1–3 g) was removed at a distance of 1 cm from the palpable tumor for the dosage of POPs and stored at the hospital Biological Resources Center and Tumor Bank Platform (BB-0033-00063). Nodal status was obtained for each patient. Data concerning: i) demographic characteristics (parity, menopausal status, age at diagnosis, work, smoking status, body mass index [BMI]), ii) tumor characteristics (hormonal receptors, her2 status, tumor grade, Ki67, tumor size), and iii) tumor extension (lymph node status, metastasis) were assessed by gynecologists specialized in oncology using standard protocols. Smoking was classified as "former, current, never". The year of the start of smoking and the number of cigarettes per day were collected for current smokers and the number of years when the patient smoked was gathered for past smokers. Patient data were anonymized and recorded on a computerized database.

2.3. Chemical measurement

The POPs were measured in adipose tissue for each patient. The isolation, detection and quantification of the targeted POPs was measured using ultra-trace methods based on gas chromatography (Agilent 7890A) coupled to high-resolution mass spectrometry (GC-HRMS) as previously described elsewhere (Koual et al., 2019). Forty-two POPs were measured, among them 17 dioxins (PCDD/F), 16 polychlorobiphenyls (PCB), 8 polybromodiphenylethers (PBDE) and 2,2′,4,4′,5,5′-hexabromobiphenyl (PBB153). The complete list can be found in Supplemental Table S1. PCBs were separated in dioxin-like and non-dioxin like PCBs.

2.4. Statistical analysis

Demographic characteristics were summarized using mean and standard deviation (SD), as well as median and interquartile range (IQR) for continuous variables and frequency and percentage for categorical variables. Continuous variables were log-transformed, centered to the mean, and scaled to the SD. Comparison of variables between cancer groups were performed with Mann–Whitney *U* test for continuous data and Fisher's exact test for categorical data. Unsupervised analysis such as principal component analysis (PCA) was used to characterize the chemical mixture structure. Spearman's Rank-Order correlation analysis was used to assess the bivariate associations between chemicals in the ensemble of participants and stratified considering the metastatic cancer status. In addition, principal component analysis was used to explore the overall data structure and the projection of observations.

The main hallmarks of cancer aggressiveness were used as outcomes in the multivariate regression analysis and multipollutant models: presence of metastatic breast cancer (yes/no, binary), tumor size (>20 mm/<20 mm, binary), tumor size (mm, continuous). All models were adjusted for the confounding variables: age, BMI, smoking, menopause, and family history of breast cancer.

Single-pollutant models were fit using logistic and lineal multivariate regressions. For the main analysis, we used two complementary multipollutant approaches: the WQS and BKMR models. The WQS models were built using the "Gaussian" and "binomial" link function in positive and negative directionality mode with the R package "*gWQS*" v 4.04.4. Under the assumption of homogeneity of direction of effects and no interactions, WQS compute overall scoring weights for positive and negative directions with the relative weight of each chemical. Considering the modest sample size, the model was fitted to the full dataset without splitting, as previously suggested, through 1000 iterations (Carrico et al., 2015). Bayesian Kernel Machine Regression (BKMR) was conducted with R package '*bkmr*' v4.0.4 using the *probit* (binomial) link function for binary outcomes and "Gaussian" function for continuous outcomes considering default prior parameters and 10,000 iterations to ensure convergence (Bobb et al., 2015). Considering the high correlation between POPs from certain families, different strategies were used for variable selection including a) the component-wise approach, b) a hierarchical variable selection grouped by chemical families, c) a hierarchical variable selection grouped by Spearman's Rank correlation coefficients. In addition, the analyses were conducted specifically for

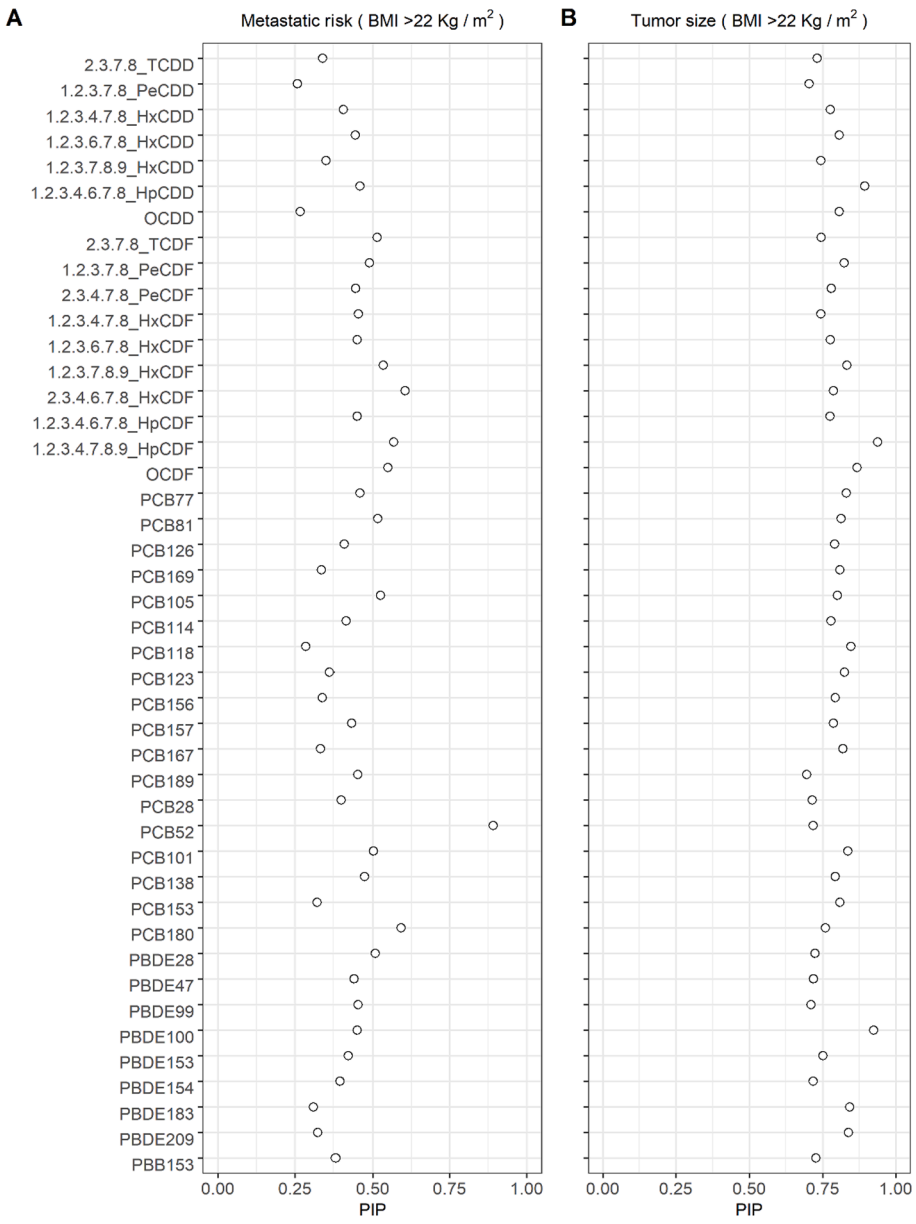

Fig. 5. Identification of relevant chemicals in the mixture using the Bayesian Kernel Machine Regression models (BKMR). Scatter plot depicting the posterior inclusion probabilities (PIP) of persistent organic pollutants included in the BKMR models conducted with hierarchical variable sectioned for the subset with body mass index (BMI) above 22 kg/m^2 for metastatic risk (A) or tumor size (B). The PIP can be interpreted as the relative influence of each variable in the model.

each chemical family and stratified by BMI (BMI $>$ 22 kg/m^2). This threshold of BMI was set at 22 kg/m^2 in order to ensure a minimal sample of observations to converge the models. All analyses were conducted with R software version 4.0.2.

3. Results

3.1. Characteristics of participants

Out of ninety-one females with breast cancer included, 38 presented a metastasis (Table S2). For the whole population, the mean age was 62 years old (+/- 14 years old, range 37–93), 72.5 % were menopaused (66/91) and the mean BMI was 24.5 kg/m^2 (+/- 4.1, range 17–34). Concerning tumor characteristics, the mean tumor size was 25.4 mm (+/- 14.6 mm, range: 4–80 mm), the majority of patients had tumors measuring $>$ 2 cm (52,7%) and most had an invasive ductal carcinoma (78 %). The most frequent immunohistochemistry finding was luminal B (53/91), followed by luminal A (24/91), triple negative (11/91) and Her2+ (3/91) cancers (Prat et al., 2015). Distributions of POPs in adipose tissue of these patients have been extensively reported elsewhere (Koual et al., 2019).

3.2. Single pollutant models

As previously reported (Koual et al., 2019), the single pollutant models globally showed lack of associations between POPs and the odds of presenting a metastatic breast cancer or a tumor size higher than 20 mm (Fig. 1), except for 2.3.7.8-TCDD, positively associated with tumor size (Fig. 1C and 1D). For the sub-group of women with body mass index above 22 kg/m^2, significant associations were found between 2.3.7.8-TCDD, some PCBs (156, 157, 167, 169 and 189) and PBDE153 with metastatic risk, suggesting a potential modification effect due to the adiposity (Fig. 1B).

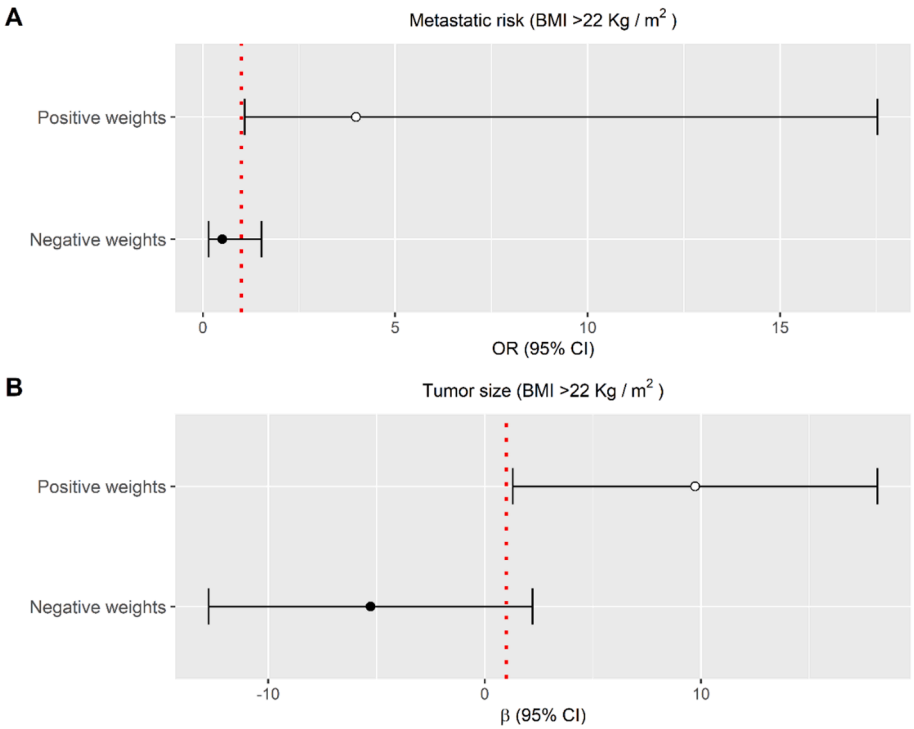

Fig. 6. Overall associations of the mixture with metastatic risk for women with a body mass index (BMI) above 22 kg/m^2 using the weighted quantile sum regression for metastatic risk (A) or tumor size (B) for women with body mass index (BMI) above 22 kg/m^2. Statistically significant associations are highlighted with white circles.

3.3. Unsupervised chemical mixture characterization

Strong positive correlations (rho > 0.6) are present between most PCDD/Fs and PCBs with some minor exceptions showing mild or no correlations, such as OCDF or 1.2.3.4.7.8.9 HpCDF (Fig. 2). Interestingly, the congeners from groups of PBDEs were not correlated with the rest of POPs, except for PBB153 that correlated with PCBs (180 and 189). A similar correlation pattern of POPs in adipose tissue was observed among the group of patients with metastatic and non-metastatic breast cancer as previsouly published (Koual et al., 2019) Supplemental Fig. S1. Concentrations of dioxin-like toxic equivalents (TEQ) were slightly higher among women with non-metastatic cancer, who in turn, presented lower concentrations of non-dioxin-like PCBs and brominated flame retardants (BFRs) (Fig. 3A-C). The relative abundance of congeners was similar among cancer groups for most congeners except for PBDE209 and PBDE47, slightly higher and lower in the metastatic group, respectively (Fig. 3C). The principal component analysis showed clear overlap of patients based on cancer type (Fig. S2A). In turn, the hierarchical clustering based on those principal components showed that main discrimination was driven by age (Fig. S1B). Similar results were found in the sub-group of patients with a BMI > 22 kg/m^2 (results not shown).

3.4. Identification of chemicals with major contribution in the mixture

The chemicals with major contribution to the global association of the mixture were identified using the WQS and BKMR models. For the WQS regression model, pollutants are ranked by the probability that the chemical has the most weight in the mixture and therefore an important role. They are separated in positive and negative weights. Details are presented in Fig. 4 for metastatic risk and tumor size in the sub-group of patients with a body mass index above 22 kg/m^2. The top three pollutants most relevant for metastatic risk were PBDE 209, PCB 180 and PCB 167 for positive weights and PBB 153, PCB 123 and 1.2.3.7.8.9-HxCDF for negative weights (Fig. 4A). For tumor size, the top three pollutants most relevant were OCDF, 1.2.3.4.7.8-HxCDD and PBDE 209 for positive weights and PBDE 183, 1.2.3.4.7.8.9-HpCDF and PBDE 100 for negative weights (Fig. 4B). Results for metastatic risk and tumor size from all participants are presented Supplemental Figs. S2 and S3 .

Using the BKMR model, relevant chemicals are defined by their global weights and are identified without the type of association (positive or negative weights). The posterior inclusion probability (PIP) is used and can be interpreted as the relative influence of each variable in the model. PCB 52, PCB 180 and 1.2.3.6.7.8-HxCDF were the top three most relevant chemicals for metastatic risk and PBDE 100, 1.2.3.4.8.9-HpCDF and 1.2.3.4.6.7.8-HpCDD for tumor size in the sub-group of patients with a BMI above 22 kg/m^2 (Fig. 5). Results for all participants are presented in Supplemental Fig. S4.

3.5. Overall effect estimation

For the WQS model, the overall mixture effect of chemicals with a positive weight were significantly associated with metastatic risk for the sub-group of patients with a BMI > 22 kg/m^2 with an OR = 3.98 95 %CI = 1.09–17.53 (Fig. 6A). The mixture of pollutants with a negative weight were not significantly associated with metastatic risk. The overall mixture effect of chemicals with a positive weight were also significantly associated with tumor size for the sub-group of patients with BMI > 22 kg/m^2 with an OR: 9.73 95 %CI: 1.30–18.15 (Fig. 6B). Results of the WQS model for tumor size and metastatic risk for the entire group of patients are presented Supplemental Fig. S5.

For the BKMR model, the mixture had a non-significant association with metastatic risk in the sub-group of patients with BMI > 22 kg/m^2 (Fig. 7A) and no association for tumor size (Fig. 7B). Results for tumor size and metastatic risk for the entire group of patients are presented in Supplemental Fig. S7. In a secondary analysis, chemicals were then separated into different chemical classes: dioxins (all dioxins and dioxin-like compounds), PCDD, PCDF, dioxin-like PCB, non-dioxin like PCB and brominated flame retardants in order to identify group-specific associations. For the group of PCDDs and all dioxins/dioxin-like compounds a

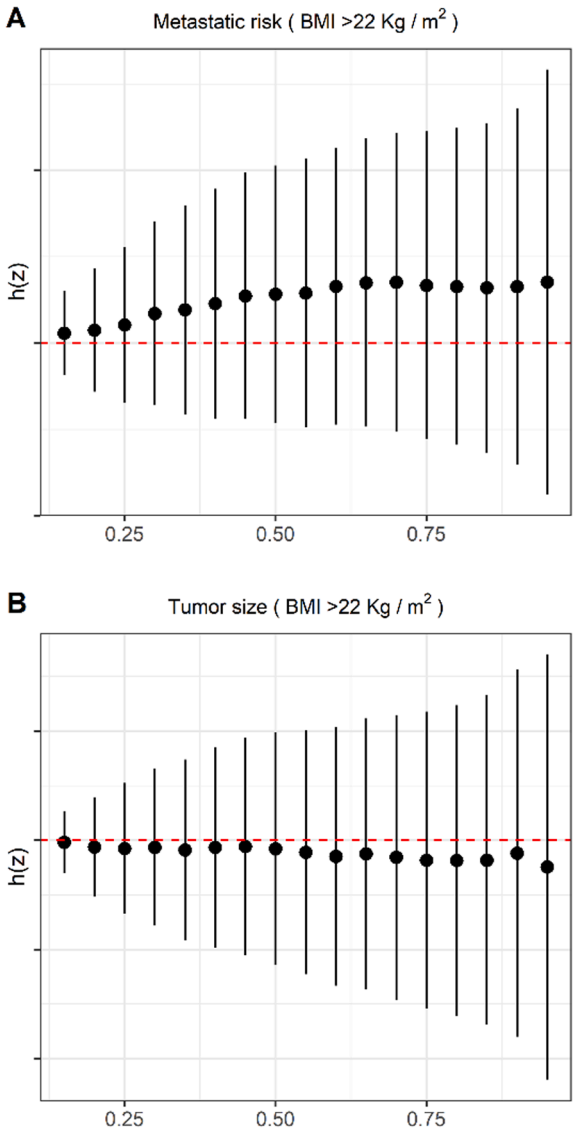

Fig. 7. Overall associations of the mixture (percentile increase) with metastatic risk for women with a body mass index (BMI) above 22 kg/m^2 using the Bayesian Kernel Machine Regression models (BKMR). The plots display the estimated change on metastatic risk (A) or tumor size (B) and respective 95 % credible intervals when all chemicals are at specific percentiles (x-axis) compared to the lowest (10th) quantile. The most influential chemicals of the mixtures for metastatic risk are displayed in Fig. 8 were the posterior inclusion probabilities computed with the hierarchical variable selection are displayed.

significant association between the cumulative exposure to the mixture with metastatic risk was observed only for the middle quantiles (Fig. 8). The rest of results for tumor size and metastatic risk for the presented Supplemental Fig. S7-9.

3.6. Interactions

The graphical visualization of interactions between chemicals estimated from the BKMR models did not suggest statistical interactions (Supplemental Fig. S11-14).

4. Discussion

To the best of our knowledge, this is the first study attempting to explore the associations between breast cancer aggressiveness and mixtures of pollutants in the adipose tissue surrounding breast tumors. The results with the entire group of participants did not reveal statistically significant associations with metastatic risk or tumor size. Nonetheless, some associations were found between mixtures of POPs and tumor size and metastasis in the sub-group of patients with a BMI > 22 kg/m^2, mainly driven by dioxins and chemical homologues.

The analysis of mixtures of POPs are especially challenging due to the high correlation between compounds demanding the use of advanced computational methods. We applied two complementary models, WQS and BKMR, allowing to address major questions about chemical mixtures (Gibson et al., 2019; Lazarevic et al., 2019). The results from both methods provide specific information valuable for this analysis (Guillien et al., 2021). In both cases, we found a modifying effect of the BMI when we focused the analysis, excluding women with lowest adiposity. We were not surprised to find different results between the two models. The WQS assesses the overall positive and negative effects of the mixture and identifies the most relevant pollutants with an easy and visual interpretation of the results. “Good” and “bad” actors are identified, and the overall weight of the mixture is evaluated (Carrico et al., 2015). The main setback of this model is that is does not consider interactions between pollutants, only additivity. The BKMR model also assesses the overall effect of the mixture (Bobb et al., 2015). The advantage is that it evaluates interactions between pollutants and non-linear associations. This model provided specific information about the exposure–response shape, suggesting a non-linear effect of the mixtures and allowed the exploration of interactions.

Using the WQS regression, the three pollutants with the highest positive weights were PBDE 209, PCB 180 and PCB 167 for metastatic risk and OCDF, 1.2.3.4.7.8.HxCDD and PBDE 209 for tumor size in the sub-group of patients with a BMI > 22 kg/m^2. The overall effect of the mixture was associated with metastatic risk and tumor size. In the BKMR model, PCB 52, PCB 180 and 1.2.3.6.7.8-HxCDF were the most relevant chemicals for metastatic risk and PBDE 100, 1.2.3.4.8.9-HpCDF and 1.2.3.4.6.7.8-HpCDD for tumor size in this sub-group of women. Our findings are consistent with previously published epidemiological and toxicological studies. For instance, PBDE 209, frequently highlighted across models and outcomes, presents estrogen-like activities and has a pro-tumor effect in breast cancer cell models probably due to an immunosuppressing effect (Barber et al., 2006; Li et al., 2012). Likewise, several PCBs were recurrent in our mixtures. This vast family of compounds is known for its dioxin-like activity for several POPs, their potential for inducing cytochrome P450 enzymes or estrogenicity (McFarland and Clarke, 1989; Wolff et al., 1997) but also their suspected role in breast cancer (Wolff et al., 1997; Soto et al., 1995; Pěnčíková et al., 2018). However, the overall evidence is inconclusive (Brody et al., 2007; Negri et al., 2003; Moysich et al., 2002). Only few studies have evaluated the metastatic and recurrence risk for breast cancer and PCB 153 was found to be predictive of lymph node invasion (OR 2.12; 95 % CI,1.05–4.30; third tercile versus first) (Demers et al., 2000) and PCBs in *peri*-tumoral adipose tissue were associated with an increased risk of breast cancer recurrence (OR 2.9; 95 % CI, 1.02–8.2) (Muscat et al., 2003). In turn, patients with ER-positive cancers with the highest sum of serum PCB, had a higher risk of breast cancer death (OR 2.5, 95 % CI, 1.1–5.7) (Høyer et al., 2001). PCB 180, associated with metastatic risk using the BKMR and WQS models has already been correlated to breast cancer incidence and aggressiveness (Høyer et al., 2001; Holford et al., 2000; He et al., 2017). To date, no previous studies have conducted multipollutant analysis of associations between organochlorinated POPs and breast cancer metastasis risk. However, a multipollutant analysis on breast cancer risk using a penalized ridge regression revealed PCB congener-specific effects, either protective or adverse, whereas total PCBs were not associated with breast risk (Holford et al., 2000). Moreover, the relevance of the susceptibility window of exposure was also highlighted by Cohn et al., who found an association between exposure to PCB 167, 187 and 203 in the post-partum period and an increased risk of breast cancer incidence after a follow-up of 17 year on average (Cohn et al., 2012). In our analysis by chemical family, using the BKMR model,

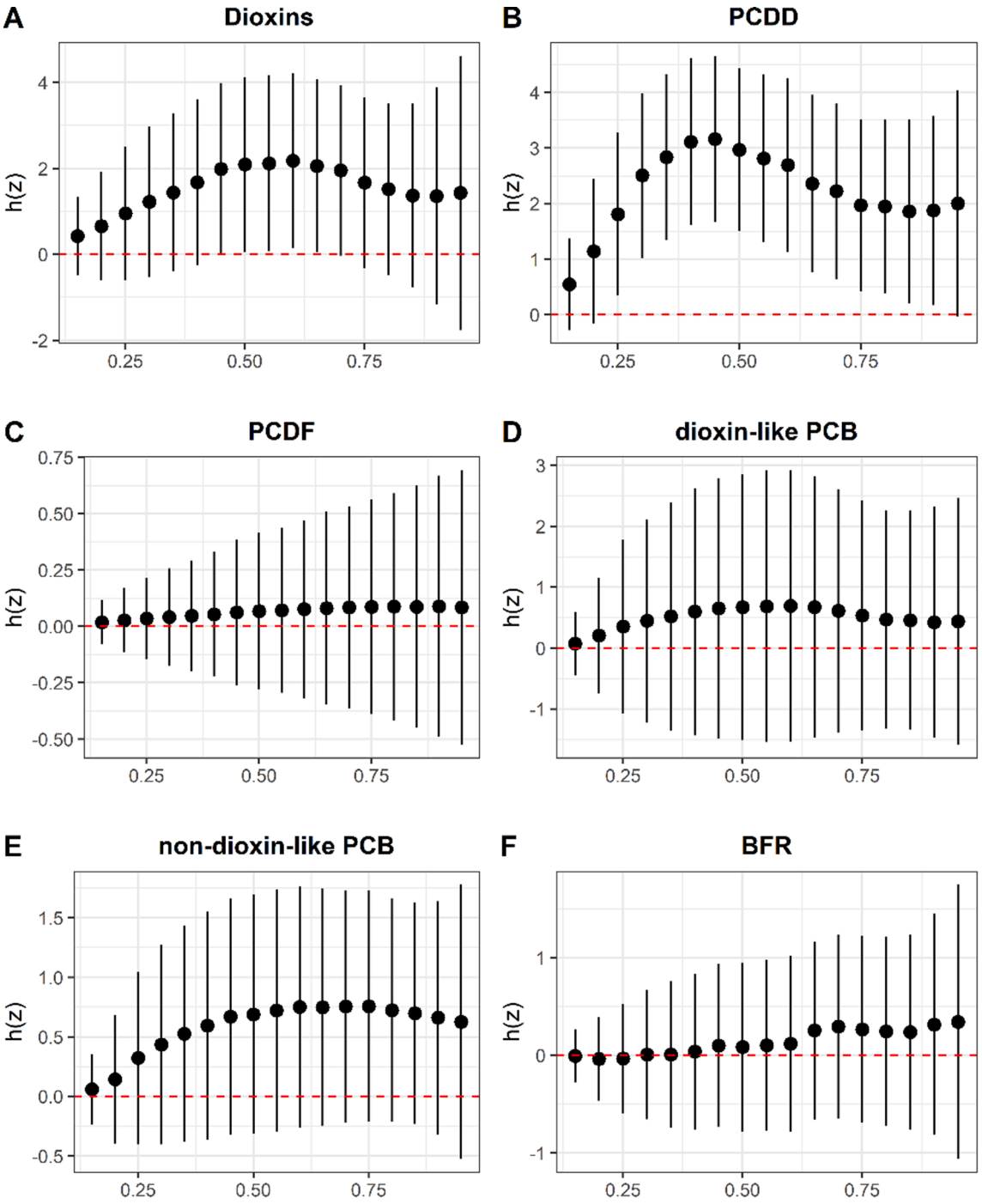

Fig. 8. Joint associations of persistent organic pollutant mixtures with metastatic risk for women with body mass index (BMI) above 22 kg/m^2 estimated with Bayesian kernel machine regression models for the different chemical families. Panel A displays the effect of all of dioxins and dioxin-like polychlorinated biphenyls (PCB); Panel B, the sub-group of polychlorinated dibenzodioxins (PCDD); Panel C; the sub-group of polychlorinated dibenzofurans (PCDF), Panel D the group of dioxin-like PCB; Panel E, non-dioxin-like PCB and Panel F, the group of brominated flame retardants. The plots display the estimated change on metastatic risk and respective 95 % credible intervals when all chemicals are at specific percentiles (x-axis) compared to the lowest (10th) quantile.

we found that the group of PCDD and dioxins were associated with a nonlinear association with metastatic risk. Dioxins are a group of highly toxic pollutants, whose disruptive roles in immunological, endocrinological, lymphatic-hematopoietic cancer and reproductive diseases, has been extensively studied (Eskenazi et al., 2018. Epidemiologic studies have reported associations between exposure to dioxins and breast cancer mortality (Manz et al., 1991; Manuwald et al., 2012; Revich et al., 2001).

Some *in vitro* studies have also evaluated the impact of POPs mixtures on cancer cell proliferation or estrogenicity with diverging results (depending on cell type, estrogenicity, pollutants used…) (Shan et al., 2020; Aubé et al., 2011; Arcaro et al., 1998; Rajapakse et al., 2002; Silva et al., 2002). For instance, a complex mixture of 15 organochlorines increased proliferation in MCF-7 cells (ER-positive) whereas it decreased proliferation in triple negative MDA-MB −231 cells and had no effect on ER-positive T47D cells (Aubé et al., 2011). Our analysis also showed that some congeners like PCB28 were inversely associated with metastasis risk, thus suggesting a "protective" effect for some POPs. The inverse

associations between low-chlorinated PCBs and breast cancer risk have been previously reported, mostly for PCB28 and PCB52 (Huang et al., 2019) but also PCB153 (Bachelet et al., 2019). Some authors have attributed these unexpected associations to the statistical instability of models (i.e. small changes on input parameters may have large influence on final estimates), caused by co-linearity (Holford et al., 2000). Another explanation could be due to the overexpression of cytochrome P450 2B (CYP2B) among metastatic cases with respect to non-metastatic cases. This enzyme is induced by a large number of PCBs and dioxins and is the main enzyme catalyzing the low chlorinated PCBs metabolism into reactive quinones (Maldonado-Rojas et al., 2016; Lin et al., 2009). Hence, it may be plausible that high CYP2B inducers would present the lowest concentrations of low-chlorinated PCBs, however this must be confirmed in experimental studies. This also raises several questions regarding the exposure to other contaminants, drugs or food components which are able to induce CYP2B and subsequently to larger mixtures of chemicals.

The results of this exploratory study should be considered taking into account some limitations. First, the small size of our sample of participant which can impair the stability of our estimates, especially regarding the specific congeners selected as main contributors of the overall associations. In order to evaluate the stability of our findings, we re-ran the models, multiple times (e.g. *seeds*), finding consistent results across the entire sets. Under high correlation data-sets, there is a risk of selecting a wrong biomarker highly correlated to the real one if the first one has lower instrumental error associated (Gibson et al., 2019; Pollack et al., 2013). Unfortunately, this issue cannot be alleviated with larger sample size (Agier et al., 2016), but eventually expert toxicological knowledge can be used to inform the congeners selection. Novel statistical methods incorporating that a priori toxicological information in the model (McGee et al., 2022), will be of high utility to integrate the consolidated AOP framework information of breast cancer metastasis (Benoit et al., 2022), in multipollutant analysis. In addition, other multipollutant models are currently available including extending WQS in a Bayesian framework (Colicino et al., 2020) or overcoming the assumption of directional homogeneity through quantile g-computation and outperforming WQS in some scenarios (Keil et al., 2020). Second, adipose tissue sampling was carried out once and at time of surgery due to the cost and invasiveness, thus reverse causality cannot be completely ruled out. For instance, drastic weight loss can lead to increased serum POPs and to a significant 15 % decrease in total PCB body burden (Kim et al., 2011). In our case, in order to prevent the potential impact of weight loss during the diagnostic period of cancer (Fénichel et al., 2021), we preferred the use of adipose tissue over serum. Also, inherent to any observational study, some residual confounding could persist, despite the fact that the models were adjusted for age, BMI, history of breast cancer, smoking and menopausal status based on the current literature (Brody et al., 2007; Zhang et al., 2004; Laden et al., 2002). Other confounding factors tested (breastfeeding, parity, professional category, living area, hormonal contraception) were not pertinent here..Finally, the BMI cut-off of 22 kg/m^2 used here for computational convenience, may appear unorthodox since overweight is defined as a BMI $>$ 25 kg/m^2. However, it is noteworthy, that conventional BMI cutoffs from general population may not be sensitive to classify adiposity sub-groups among older population above 60 years ols with an uncertainty ranging between 1 and 5 BMI points depending on the studies (Batsis et al., 2016; Donini et al., 2020). Moreover, even if a BMI above 25 may be predictive of cardiovascular diseases may it does not mean that it should be predictive of environmentally related diseases/effects.

In conclusion, this exploratory study supports the interest of using multipollutant statistical methods to gain insight on the relationships between POPs and metastasis risk. The results support that mixtures of dioxin-like pollutants may be associated with metastasis and tumor size in patients with a BMI $>$ 22 kg/m^2. These clinical results are coherent with *in vitro* studies (Koual et al., 2021). Despite several low-chlorinated PCB congeners being negatively associated with metastatic risk, interactions between POPs were not identified. These findings urge to conduct a study with a larger sample size in order to validate these findings.

5. Fundings

This work was supported by PNRPE (METAPOP project, n° 11-MRES-PNRPE-5-CVS-031 Chorus n°2100532530); Institut National de la Santé et de la Recherche Médicale (INSERM); Université Paris Cité (UPCité); Assistance Publique des Hôpitaux de Paris (APHP).

CRediT authorship contribution statement

Louise Benoit: Conceptualization, Formal analysis, Methodology, Validation, Visualization, Writing – original draft. **Meriem Koual:** Conceptualization, Data curation, Funding acquisition, Supervision, Writing – review & editing. **Céline Tomkiewicz:** Conceptualization, Supervision, Writing – review & editing. **Anne-Sophie Bats:** Conceptualization, Supervision, Writing – review & editing. **Jean-Philippe Antignac:** Conceptualization, Supervision, Writing – review & editing. **Xavier Coumoul:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Validation, Visualization, Writing – review & editing. **Robert Barouki:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Validation, Visualization, Writing – review & editing. **German Cano-Sancho:** Conceptualization, Formal analysis, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank Daniela Geromin from the Biological Resources Center and Tumor Bank Platform, Georges-Pompidou European Hospital (Paris, France)

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107615.

References

Agier, L., Portengen, L., Chadeau-Hyam, M., Basagaña, X., Giorgis-Allemand, L., Siroux, V., et al., 2016. A Systematic Comparison of Linear Regression-Based Statistical Methods to Assess Exposome-Health Associations. Environ. Health Perspect. 124 (12), 1848–1856.

Arcaro, K.F., Vakharia, D.D., Yang, Y., Gierthy, J.F., 1998. Lack of synergy by mixtures of weakly estrogenic hydroxylated polychlorinated biphenyls and pesticides. Environ. Health Perspect. 106 (suppl 4), 1041–1046.

Aubé, M., Larochelle, C., Ayotte, P., 2011. Differential effects of a complex organochlorine mixture on the proliferation of breast cancer cell lines. Environ. Res. 111 (3), 337–347.

Bachelet, D., Verner, M.A., Neri, M., Cordina Duverger, É., Charlier, C., Arveux, P., et al., 2019. Breast Cancer and Exposure to Organochlorines in the CECILE Study: Associations with Plasma Levels Measured at the Time of Diagnosis and Estimated during Adolescence. Int. J. Environ. Res. Public Health 16 (2), E271.

Barber, J.L., Walsh, M.J., Hewitt, R., Jones, K.C., Martin, F.L., 2006. Low-dose treatment with polybrominated diphenyl ethers (PBDEs) induce altered characteristics in MCF-7 cells. Mutagenesis 21 (5), 351–360.

Barouki, R., Audouze, K., Becker, C., Blaha, L., Coumoul, X., Karakitsios, S., et al., 2022. The Exposome and Toxicology: A Win-Win Collaboration. Toxicol. Sci. Off. J. Soc. Toxicol. 186 (1), 1–11.
Batsis, J.A., Mackenzie, T.A., Bartels, S.J., Sahakyan, K.R., Somers, V.K., Lopez-Jimenez, F., 2016. Diagnostic accuracy of body mass index to identify obesity in older adults: NHANES 1999–2004. Int. J. Obes. 40 (5), 761–767, 2005.
Benoit, L., Jornod, F., Zgheib, E., Tomkiewicz, C., Koual, M., Coustillet, T., et al., 2022. Adverse outcome pathway from activation of the AhR to breast cancer-related death. Environ. Int. 165, 107323.
Bobb, J.F., Valeri, L., Claus Henn, B., Christiani, D.C., Wright, R.O., Mazumdar, M., et al., 2015. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostat. Oxf. Engl. 16 (3), 493–508.
Bokobza, E., Hinault, C., Tiroille, V., Clavel, S., Bost, F., Chevalier, N., 2021. The Adipose Tissue at the Crosstalk Between EDCs and Cancer Development. Front. Endocrinol. 20 (12), 691658.
Braun, J.M., Gennings, C., Hauser, R., Webster, T.F., 2016. What Can Epidemiological Studies Tell Us about the Impact of Chemical Mixtures on Human Health? Environ. Health Perspect. 124 (1), A6–A9.
Brody, J.G., Moysich, K.B., Humblet, O., Attfield, K.R., Beehler, G.P., Rudel, R.A., 2007. Environmental pollutants and breast cancer: epidemiologic studies. Cancer 109 (12 Suppl), 2667–2711.
Burns, J.S., Williams, P.L., Sergeyev, O., Korrick, S., Lee, M.M., Revich, B., et al., 2011. Serum dioxins and polychlorinated biphenyls are associated with growth among Russian boys. Pediatrics 127 (1), e59–e68.
Carlin, D.J., Rider, C.V., Woychik, R., Birnbaum, L.S., 2013. Unraveling the health effects of environmental mixtures: an NIEHS priority. Environ. Health Perspect. 121 (1), A6–A8.
Carrico, C., Gennings, C., Wheeler, D.C., Factor-Litvak, P., 2015. Characterization of Weighted Quantile Sum Regression for Highly Correlated Data in a Risk Analysis Setting. J. Agric. Biol. Environ. Stat. 20 (1), 100–120.
Cohn, B.A., Terry, M.B., Plumb, M., Cirillo, P.M., 2012. Exposure to polychlorinated biphenyl (PCB) congeners measured shortly after giving birth and subsequent risk of maternal breast cancer before age 50. Breast Cancer Res. Treat. 136 (1), 267–275.
Colicino, E., Pedretti, N.F., Busgang, S.A., Gennings, C., 2020. Per- and poly-fluoroalkyl substances and bone mineral density: Results from the Bayesian weighted quantile sum regression. Environ. Epidemiol. Phila Pa. 4 (3), e092.
Demers, A., Ayotte, P., Brisson, J., Dodin, S., Robert, J., Dewailly, E., 2000. Risk and aggressiveness of breast cancer in relation to plasma organochlorine concentrations. Cancer Epidemiol. Biomark Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol. 9 (2), 161–166.
Donini, L.M., Pinto, A., Giusti, A.M., Lenzi, A., Poggiogalle, E., 2020. Obesity or BMI Paradox? Beneath the Tip of the Iceberg. Front. Nutr. 7, 53.
Drakvik, E., Altenburger, R., Aoki, Y., Backhaus, T., Bahadori, T., Barouki, R., et al., 2020. Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. Environ. Int. 134, 105267.
Eskenazi, B., Warner, M., Brambilla, P., Signorini, S., Ames, J., Mocarelli, P., 2018. The Seveso accident: A look at 40 years of health research and beyond. Environ. Int. 121 (Pt 1), 71–84.
Fénichel, P., Coquillard, P., Brucker-Davis, F., Marchand, P., Cano-Sancho, G., Boda, M., et al., 2021. Sustained bloodstream release of persistent organic pollutants induced by extensive weight loss after bariatric surgery: Implications for women of childbearing age. Environ. Int. 151, 106400.
Gibson, E.A., Nunez, Y., Abuawad, A., Zota, A.R., Renzetti, S., Devick, K.L., et al., 2019. An overview of methods to address distinct research questions on environmental mixtures: an application to persistent organic pollutants and leukocyte telomere length. Environ. Health Glob Access Sci. Source 18 (1), 76.
Globocan 2012 - Home [Internet]. [cited 2018 Mar 22]. Available from: http://globocan.iarc.fr/Default.aspx.
Guillien, A., Cadiou, S., Slama, R., Siroux, V., 2021. The Exposome Approach to Decipher the Role of Multiple Environmental and Lifestyle Determinants in Asthma. Int. J. Environ. Res. Public Health 18 (3), 1138.
He, Y., Peng, L., Huang, Y., Peng, X., Zheng, S., Liu, C., et al., 2017. Association of breast adipose tissue levels of polychlorinated biphenyls and breast cancer development in women from Chaoshan, China. Environ. Sci. Pollut. Res. Int. 24 (5), 4778–4790.
Holford, T.R., Zheng, T., Mayne, S.T., Zahm, S.H., Tessari, J.D., Boyle, P., 2000. Joint effects of nine polychlorinated biphenyl (PCB) congeners on breast cancer risk. Int. J. Epidemiol. 29 (6), 975–982.
Høyer, A.P., Jørgensen, T., Rank, F., Grandjean, P., 2001. Organochlorine exposures influence on breast cancer risk and survival according to estrogen receptor status: a Danish cohort-nested case-control study. BMC Cancer. 1, 8.
Huang, W., He, Y., Xiao, J., Huang, Y., Li, A., He, M., et al., 2019. Risk of breast cancer and adipose tissue concentrations of polychlorinated biphenyls and organochlorine pesticides: a hospital-based case-control study in Chinese women. Environ. Sci. Pollut. Res. Int. 26 (31), 32128–32136.
Keil, A.P., Buckley, J.P., O'Brien, K.M., Ferguson, K.K., Zhao, S., White, A.J., 2020. A Quantile-Based g-Computation Approach to Addressing the Effects of Exposure Mixtures. Environ. Health Perspect. 128 (4), 47004.
Kim, M.J., Marchand, P., Henegar, C., Antignac, J.P., Alili, R., Poitou, C., et al., 2011. Fate and Complex Pathogenic Effects of Dioxins and Polychlorinated Biphenyls in Obese Subjects before and after Drastic Weight Loss. Environ. Health Perspect. 119 (3), 377–383.
Koual, M., Cano-Sancho, G., Bats, A.-S., Tomkiewicz, C., Kaddouch-Amar, Y., Douay-Hauser, N., Ngo, C., Bonsang, H., Deloménie, M., Lecuru, F., Le Bizec, B., Marchand, P., Botton, J., Barouki, R., Antignac, J.-P., Coumoul, X., 2019. Associations between persistent organic pollutants and risk of breast cancer metastasis. Environ. Int. 132, 105028.
Koual, M., Tomkiewicz, C., Cano-Sancho, G., Antignac, J.P., Bats, A.S., Coumoul, X., 2020. Environmental chemicals, breast cancer progression and drug resistance. Environ. Health Glob. Access. Sci. Source. 19 (1), 117.
Koual, M., Tomkiewicz, C., Guerrera, I.C., Sherr, D., Barouki, R., Coumoul, X., 2021. Aggressiveness and Metastatic Potential of Breast Cancer Cells Co-Cultured with Preadipocytes and Exposed to an Environmental Pollutant Dioxin: An in Vitro and in Vivo Zebrafish Study. Environ. Health Perspect. 129 (3), 37002.
Laden, F., Ishibe, N., Hankinson, S.E., Wolff, M.S., Gertig, D.M., Hunter, D.J., et al., 2002. Polychlorinated biphenyls, cytochrome P450 1A1, and breast cancer risk in the Nurses' Health Study. Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol. 11 (12), 1560–1565.
Lazarevic, N., Barnett, A.G., Sly, P.D., Knibbs, L.D., 2019. Statistical Methodology in Studies of Prenatal Exposure to Mixtures of Endocrine-Disrupting Chemicals: A Review of Existing Approaches and New Alternatives. Environ. Health Perspect. 127 (2), 26001.
Lee, D.H., Jacobs, D.R., Park, H.Y., Carpenter, D.O., 2017. A role of low dose chemical mixtures in adipose tissue in carcinogenesis. Environ. Int. 1 (108), 170–175.
Li, Z.H., Liu, X.Y., Wang, N., Chen, J.S., Chen, Y.H., Huang, J.T., et al., 2012. Effects of Decabrominated Diphenyl Ether (PBDE-209) in Regulation of Growth and Apoptosis of Breast, Ovarian, and Cervical Cancer Cells. Environ. Health Perspect. 120 (4), 541–546.
Lin, C.H., Huang, C.L., Chuang, M.C., Wang, Y.J., Chen, D.R., Chen, S.T., et al., 2009. Protective role of estrogen receptor-alpha on lower chlorinated PCB congener-induced DNA damage and repair in human tumoral breast cells. Toxicol. Lett. 188 (1), 11–19.
Maldonado-Rojas, W., Rivera-Julio, K., Olivero-Verbel, J., Aga, D.S., 2016. Mechanisms of interaction between persistent organic pollutants (POPs) and CYP2B6: An in silico approach. Chemosphere 159, 113–125.
Manuwald, U., Velasco Garrido, M., Berger, J., Manz, A., Baur, X., 2012. Mortality study of chemical workers exposed to dioxins: follow-up 23 years after chemical plant closure. Occup. Environ. Med. 69 (9), 636–642.
Manz, A., Berger, J., Dwyer, J.H., Flesch-Janys, D., Nagel, S., Waltsgott, H., 1991. Cancer mortality among workers in chemical plant contaminated with dioxin. Lancet Lond. Engl. 338 (8773), 959–964.
McFarland, V.A., Clarke, J.U., 1989. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ. Health Perspect. 81, 225–239.
McGee, Glen, Wilson, Ander, Coull, Brent A., Webster, Thomas F., 2022. Integrating Biological Knowledge in Kernel-Based Analyses of Environmental Mixtures and Health. arxiv [Internet]. 2022 Mar; Available from: https://arxiv.org/abs/2204.00040.
Moysich, K.B., Menezes, R.J., Baker, J.A., Falkner, K.L., 2002. Environmental exposure to polychlorinated biphenyls and breast cancer risk. Rev. Environ. Health 17 (4), 263–277.
Muscat, J.E., Britton, J.A., Djordjevic, M.V., Citron, M.L., Kemeny, M., Busch-Devereaux, E., et al., 2003. Adipose concentrations of organochlorine compounds and breast cancer recurrence in Long Island, New York. Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol. 12 (12), 1474–1478.
Negri, E., Bosetti, C., Fattore, E., La Vecchia, C., 2003. Environmental exposure to polychlorinated biphenyls (PCBs) and breast cancer: a systematic review of the epidemiological evidence. Eur. J. Cancer Prev. Off. J. Eur. Cancer Prev. Organ ECP. 12 (6), 509–516.
Pěnčíková, K., Svržková, L., Strapáčová, S., Neča, J., Bartoňková, I., Dvořák, Z., et al., 2018. In vitro profiling of toxic effects of prominent environmental lower-chlorinated PCB congeners linked with endocrine disruption and tumor promotion. Environ. Pollut. Barking Essex 1987 (237), 473–486.
Pollack, A.Z., Perkins, N.J., Mumford, S.L., Ye, A., Schisterman, E.F., 2013. Correlated Biomarker Measurement Error: An Important Threat to Inference in Environmental Epidemiology. Am. J. Epidemiol. 177 (1), 84–92.
Prat, A., Pineda, E., Adamo, B., Galván, P., Fernández, A., Gaba, L., et al., 2015. Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast Edinb Scotl. 24 (Suppl 2), S26–S35.
Price, P.S., Han, X., 2011. Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment. Int. J. Environ. Res. Public Health 8 (6), 2212–2225.
Rajapakse, N., Silva, E., Kortenkamp, A., 2002. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ. Health Perspect. 110 (9), 917–921.
Revich, B.A., Sotskov, I.P., Kliuev, N.A., Brodskiĭ, E.S., Lipchenko, I.N., Muzurov, I.V., et al., 2001. Dioxins in the environment and in the blood and breast milk of residents of the town of Chapayevsk. Gig. Sanit. 6, 6–11.
Shan, A., Leng, L., Li, J., Luo, X.M., Fan, Y.J., Yang, Q., et al., 2020. TCDD-induced antagonism of MEHP-mediated migration and invasion partly involves aryl hydrocarbon receptor in MCF7 breast cancer cells. J. Hazard. Mater. 5 (398), 122869.
Siegel, R.L., Miller, K.D., Fuchs, H.E., Jemal, A., 2021. Cancer Statistics, 2021. CA Cancer J. Clin. 71 (1), 7–33.
Silva, E., Rajapakse, N., Kortenkamp, A., 2002. Something from "nothing"–eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ. Sci. Technol. 36 (8), 1751–1756.
Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N., Serrano, F.O., 1995. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ. Health Perspect. 103 (Suppl 7), 113–122.

Sun, Y.-S., Zhao, Z., Yang, Z.-N., Xu, F., Lu, H.-J., Zhu, Z.-Y., Shi, W., Jiang, J., Yao, P.-P., Zhu, H.-P., 2017. Risk Factors and Preventions of Breast Cancer. Int. J. Biol. Sci. 13 (11), 1387–1397.

Wolff, M.S., Camann, D., Gammon, M., Stellman, S.D., 1997. Proposed PCB congener groupings for epidemiological studies. Environ. Health Perspect. 105 (1), 13–14.

Woodruff, T.J., Zota, A.R., Schwartz, J.M., 2011. Environmental chemicals in pregnant women in the United States: NHANES 2003–2004. Environ. Health Perspect. 119 (6), 878–885.

Zhang, Y., Wise, J.P., Holford, T.R., Xie, H., Boyle, P., Zahm, S.H., et al., 2004. Serum polychlorinated biphenyls, cytochrome P-450 1A1 polymorphisms, and risk of breast cancer in Connecticut women. Am. J. Epidemiol. 160 (12), 1177–1183.