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Abbreviations:

ADI: Acceptable Daily Intake; AhR: Aromatic hydrocarbon Receptor; ANSES: Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail ; BMR: Basal Metabolic Rate ; BC: Breast Cancer; β-HCH : β-Hexachlorocyclohexane ; BMI: Body Mass Index; CépiDC : French Centre for Epidemiology Medical Causes of Death database ; CI: Confidence Interval ; CNIL: Commission Nationale de l'Informatique et des Libertés ; CVUA: Chemisches und Veterinäruntersuchungsamt; DDE: Dichlorodiphenyldichloroethylene ; DDT: Dichlorodiphenyltrichloroethane ; DNA: Deoxyribonucleic acid ; EDI: Estimated Daily Intake; EFSA: European Food and Safety Authority; ER-/PR-: Estrogen Receptor Negative/Progesterone Receptor Negative ; FFQ: Food Frequency Questionnaire; HCB: Hexachlorobenzene ; HR: Hazard Ratio; ICD-10: International Statistical Classification of Diseases and Related Health Problems 10th Revision; IPAQ: International Physical Activity Questionnaire; IRB INSERM: Institutional Review Board of the French Institute for Health and Medical Research; NMF: Non-negative Matrix Factorization; ; OC: Organochlorine; OP: Organophosphorous;; PCTA: Pentachlorothioanisole ; PNNS: Programme National Nutrition Santé; SD: Standard Deviation; SNIIRAM: Système National d'Information Inter-Régimes de l'Assurance Maladie; sPNNS-GS2: simplified Programme National Nutrition Santé Guideline Score 2; WHO: World Health Organization.

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Data Share Statement:

Data of the study are protected under the protection of health data regulation set by the French National Commission for Information Technology and Liberties (Commission Nationale de l'Informatique et des Libertés, CNIL). The data are available upon reasonable request to the study's operational manager, Nathalie Pecollo (n.pecollo@eren.smbh.univ-paris13.fr), for review by the steering committee of the NutriNet-Santé study.

Abstract:

Background:

Some pesticides, used in large quantities in current agricultural practices all over Europe, are suspected of adverse effects on human reproductive health (breast and prostate cancers), through mechanisms of endocrine disruption and possible carcinogenic properties, as observed in agricultural settings.

However, evidence on dietary pesticide exposure and breast cancer (BC) is lacking for general population. We aimed to assess the associations between dietary exposure to pesticides and BC risk among postmenopausal women of the NutriNet-Santé cohort.

Methods:

In 2014, participants completed a self-administered semi-quantitative Food Frequency Questionnaire, distinguishing conventional and organic foods. Exposures to 25 active substances used in EU plant protection products were estimated using a pesticide residue database accounting for farming practices, from Chemisches und Veterinäruntersuchungsamt Stuttgart, Germany.

Non-Negative Matrix Factorization (NMF), adapted for data with excess zeros, was used to establish exposure profiles. The four extracted NMF components' quintiles were introduced into Cox models estimating Hazard Ratio (HR) and 95% confidence interval (95% CI), adjusted for known confounding factors.

Results :

A total of 13,149 postmenopausal women were included in the analysis(169 BC cases, median follow-up=4.83 years). Negative associations between component 3, reflecting low exposure to synthetic pesticides, and post-menopausal BC risk were found (HR_{Q5}=0.57; 95%CI(0.34;0.93), p-trend=0.006). Positive association between component 1 score (highly correlated to chlorpyriphos, imazalil, malathion, thiabendazole) and postmenopausal BC risk

was found specifically among overweight and obese women (HR_{Q5}=4.13; 95%CI(1.50;11.44), p-trend=0.006). No associations were detected for the other components.

Conclusions:

These associations suggest a potential role of dietary pesticide exposure on BC risk. Further research is needed to investigate mechanisms and confirm these results in other populations.

Keywords: dietary exposure; pesticides; organic farming; epidemiology; breast cancer; environmental health.

Key Messages:

- Diet is considered as the main exposure route for pesticide exposure in the general population. Dietary pesticide exposure has been rarely studied in relation with cancers.
- Non-Negative Matrix Factorization (NMF), a method adapted for data with excess zeros, was used to characterise dietary pesticide exposure profiles.
- We observed a reduction in the risk of postmenopausal breast cancer for NMF Component 3 (reflecting low exposure to several synthetic pesticides).
- A positive association between NMF component 1 score (highly correlated to chlorpyriphos, imazalil, malathion, thiabendazole) and postmenopausal breast cancer risk was observed specifically among overweight and obese women.
- For NMF Components 2 and 4, Hazard Ratios (HR) were $HR_{\text{Quintile 5 vs Quintile 1}}$ 0.96, 95% Confidence Interval (0.59; 1.56), p for trend : 0.30 and $HR_{\text{Quintile 5 vs Quintile 1}}$ 0.65, 95% Confidence Interval (0.38; 1.12), p for trend : 0.13.

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Introduction:

Large quantities of plant protection products are used in current European agricultural practices [1]. In particular, France has high usage of pesticides, synthetic or natural, both in global tonnages (80 000 tons in 2018) and by surface area (4.45 kg/ha in 2018) [2–5].

Deleterious impacts of pesticides on human health have been evidenced. Various effects of pesticide active substances have been documented , including genetic material alteration, endocrine disrupting effects, cell apoptosis and cell signaling dysregulation, and oxidative stress induction [4,6–8]. These mechanisms have been shown to be involved in carcinogenesis [9]. Recently, IARC classified many pesticides as "probably carcinogenic to humans" (Group 2A) and "possibly carcinogenic to humans" (Group 2B) [10]. In addition, many pesticides exhibit endocrine disruptors properties [7].

Indeed, cancer is nowadays the first or second leading cause of premature death in many European countries. It is the first cause of mortality in France [11–13], breast cancer being the most common and leading cause of cancer death for women in France. Associations between occupational pesticide exposure in agricultural settings (involving respiratory and cutaneous exposure routes) and the occurrence of some locations of cancers (myeloma, non-Hodgkin lymphoma, prostate) were found in several studies[14–19]. Associations in agricultural settings with other cancer locations have been reported (stomach, esophagus, liver, colorectal…), and especially reproductive system cancers (prostate, breast), potentially induced by endocrine disruption mechanisms [4,7,20]. Notably, associations between breast cancer risk and organophosphorus pesticide exposure were found for farmer's wives in some studies [21,22]. However, in the general population, although food is considered as the first exposure pathway, data is lacking on associations between dietary exposure to pesticides and cancer [23,24]. This may be explained by three main challenges. Firstly, measuring pesticide residue concentrations in food is expensive and tedious. In addition, it is difficult to measure pesticides mixtures (opposed to compounds taken separately), but necessary, as it can

potentially lead to synergistic effects. Finally, data existing so far generally lacks of precision regarding the production system (conventional vs organic), limiting proper estimation of pesticide exposure.

Recently, a study conducted in the NutriNet-Santé cohort showed protective associations between the high proportion of organic food in the diet and different types of cancers, including postmenopausal breast cancer[22]. An hypothesis advanced to explain this association was the potentially lower concentrations of pesticides residues in plant organic foods [25].

In that context, the purpose of this work was to study the associations between dietary pesticide exposure profiles and breast cancer risk among postmenopausal women included in the NutriNet-Santé cohort.

Material and Methods:

Study population

The NutriNet-Santé study is a web-based prospective cohort of adults launched in France in May 2009 [26]. Inclusion criteria was to be aged 18 years old and over and to speak French. A set of self-administered validated questionnaires [27–29] was completed online by participants at baseline and repeated every year. Complementary questionnaires were regularly proposed concerning dietary behaviors and specific health issue during follow-up.

Dietary intake assessment

A 264-item web-based self-administered semi-quantitative food frequency questionnaire (Org-FFQ) distinguishing organic and conventional foods was sent to the participants between June and December 2014. The Org-FFQ has been extensively described elsewhere [30]. Briefly, it was elaborated on the basis of an existing validated FFQ [31] to which a 5-point ordinal scale was added to measure the frequency of organic food consumption. For each item, participants provided their frequency of consumption and the quantity consumed helping with photographs showing different portion sizes [32]. For food and beverages with an existing organic version (labelled), participants answered the question "How often was the product of organic origin?" by selecting 1 of the 5 following frequency modalities: never, rarely, half-of-time, often, or always. The organic food consumption was then obtained by attributing the respective percentages, 0, 25, 50, 75, and 100, to the modalities. Weighting and sensitivity analyses for the Org-FFQ have been published elsewhere [30].

All food and beverage items were aggregated into 33 food groups. Nutritional values were obtained from a published food composition database [33]. A global proportion (as weight) of organic food in the diet was calculated as well as the proportion of organic food for each food group.

Pesticide exposure assessment

Dietary pesticide exposure was estimated by combining dietary intakes of each adult with pesticide residue concentration values in foods using contamination data from Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart, a European Union reference laboratory for pesticides [34]. The database comprised contamination data for conventional and organic food products. Twenty-five commonly used pesticides were selected among components available in this database, given both their frequency of detection above the Maximum Residue Levels (MRL) when sufficient data were available, and their frequency above Acceptable Daily Intake (ADI) otherwise, as detailed in Baudry et al. 2019 study [35]. Pesticides commonly used in organic agricultural systems (e.g. natural pyrethrins, spinosad) were also selected. These criteria made it possible to take into account a broad spectrum of classes of pesticides. The 264 Org-FFQ items were decomposed into 442 ingredients (comprising at least 5% of at least one food item). Animal-based ingredients were excluded, as CVUA encompassed plant-based ingredients only. Indeed, plant-based foods have markedly more frequent and higher pesticides residues levels than foods of animal origin [36]. The resulting 180 plant ingredients were matched to CVUA database and then were attributed a contamination value in organic and conventional farming modes (as the mean of corresponding data point). A flowchart of the different steps for the decomposition and matching is shown in **Supplementary Material 1**.

For each ingredient/pesticide pair in conventional and organic farming, a frequency of detection and a frequency of quantification were determined using the formula as follows:

$$Frequency\ of detection = 100 \times \frac{Number\ of analyses - Number\ of\ undetected}{Number\ of\ analyses}$$

$$Frequency\ of quantification = 100 \times \frac{Number\ of\ analyses - Number\ of\ unquantified}{Number\ of\ analyses}$$

Treatment of data below detection limit has been extensively described elsewhere [35].

As food consumption data from NutriNet-Santé referred to edible foods (bone-free, peeled or cooked products), edibility and cooking factors were allocated to each ingredient when necessary. The same conversion factors were used for both conventional and organic products. Cooking or peeling effects on pesticide residue levels were not accounted for as dilution factors are not available for all food/pesticide couples [37]. For each pesticide, the estimated daily intake (EDI) (in µg/kg of weight/day) under both lower- and upper-bound scenarios was calculated using methods recommended by EFSA and WHO [38,39]. Lower-bound (optimistic) scenario was used for this work, as more in line with available literature comparing both production systems [35,40].

Covariates

Baseline and yearly questionnaires collected sociodemographic and lifestyle characteristics such as sex, date of birth, occupation, educational level, smoking status, number of children. Monthly income by household unit was obtained was obtained using both the household income and composition. Anthropometric measures (height, weight), physical activity (using the validated Physical Activity Questionnaire [41]) and health status (menopausal status, family history of cancer, treatments) were also collected.

A specific questionnaire on environmental exposure collected the type of environment in which participants lived: agricultural or urban area.

The simplified Programme National Nutrition Santé Guideline Score 2 (sPNNS-GS2), based on the level of adherence to 2017 French dietary guidelines proposed by the High Council of Public Health [42,43], the provegetarian score [44] and the percentage of ultra-processed foods in the diet [45] were computed to be used as adjustment factors. Briefly, the sPNNS-GS2 includes 13 components. One point was allocated for following the guideline (and 0 otherwise), and conversely for moderation components. Component with several subcomponents were standardized and a penalty for overconsumption was applied. The score ranged from -∞ to

14.25. Component, cut-off, scoring system and ponderation are presented in **Supplementary Material 2**.

The provegetarian score was computed as follows [44]: 7 vegetable food groups and 5 animal food groups were defined and sex-specific quintiles adjusted for total energy intake were calculated. For each plant component, 1 to 5 points were allocated to quintile 1 to 5 and for animal food groups the scoring was reversed. The provegetarian score was obtained by summing each quintile value of vegetable food group and each reverse quintile value of animal food group thus ranging from 12 (low plant food consumption) to 60 (high plant food consumption).

Percentage of ultra-processed foods consumed was computed after classification of foods using NOVA categories [45], by a committee of dietitians and researchers [46]. NOVA classification is described in details in **Supplementary Material 3**. Data used to calculate the proportion of ultra-processed foods in the diet were the closest to the Org-FFQ completion date.

Cancer cases

Health events were declared by participants through a yearly health status questionnaire and a dedicated web-interface at any time of the study. All medical records were collected and analyzed by dedicated physicians. Physicians of participants declaring major health events were contacted to collect additional information if necessary. Validation of these major health events was carried out by a medical expert committee.

Overall, medical records were obtained for more than 90% of self-reported cancer cases. Moreover, we performed a linkage between our declared health data to medico-administrative registers of the national health insurance system (Système National d'Information Inter-Régimes de l'Assurance Maladie [SNIIRAM] databases). Mortality data were also used from the French Centre for Epidemiology Medical Causes of Death database (CépiDC). Cancer

cases were classified using the *International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Clinical Modification* [47]. In this study, we considered all first primary breast cancers (ICD-10 C50) diagnosed between baseline (i.e. the date of completion of the Org-FFQ in 2014 or the menopause date, whichever occurred last) and 18 July 2019 to be cases.

Statistical analyses

A flowchart for the study sample selection is presented in Figure 1.

For the present study, postmenopausal female participants who completed the Org-FFQ between June and December 2014 (N = 28,445), with no missing covariates for basal metabolic rate computation (N =28,137), who were not detected as under- or over-reporters (N = 27,158), who were postmenopausal and free of breast cancer when they completed the Org-FFQ, were selected (N=13,149).

Regarding under or overreporters, only participants with a plausible energy intake were included in the analyses. The detection method for under and overreporters was based on the comparison between energy intake and energy requirement and is extensively described in a previous article by Baudry et al. [30]

Dietary pesticide exposure profiles were analyzed using Non-Negative Matrix factorization (NMF) (detailed in **Supplementary Material 4**), specially adapted for non-negative data with excess zeros, developed by Lee et al [48]. In total, four components were computed for the NMF procedure using 25 selected pesticide exposure values, reflecting various pesticide exposure patterns.

Sociodemographic and lifestyle characteristics were compared between cases and non-cases, and also across NMF-extracted component quintiles using Chi^2, Mantel-Haenzel, Wilcoxon and Kruskal-Wallis tests, as appropriate.

Associations between dietary pesticide exposure, using NMF components divided into quintiles (first quintile used as reference) and breast cancer were assessed using Cox proportional hazards regression models. Participants contributed person time until the date of diagnosis of cancer, the date of last completed questionnaire, the date of death, or 18th July 2019, whichever occurred first.

NMF component scores were divided into quintiles and introduced into separate models, with age as time scale, and first quintile as reference.

Cox models were adjusted for known confounders such as smoking status, alcohol intake, educational level, physical activity (measured with International Physical Activity Questionnaire), Body Mass Index (BMI), height, family history of cancer, menopausal treatment and parity and overall quality of the diet (measured by the PNNS-GS2 score [43]).

Interactions between potential modulating factors and components were tested by introducing the multiplicative interaction term into the models, namely body mass index, sPNNS-GS2 (overall nutritional quality of the diet) and the level of plant-based consumption (using the provegetarian score). Interactions with $p<0.10$ were further investigated.

Schoenfeld residuals were used to test the proportional hazard assumption of the Cox model. Potential nonlinear effects of continuous exposure variables were evaluated using martingale residuals.

Tests for linear trend were performed using quintiles of the NMF components as ordinal variables.

Sensitivity analyses were carried out. A model was performed excluding early cases (1 year after baseline) and two other models were computed with additional adjustments for the level of ultra-processed foods in the diet, and the provegetarian score. Two-sided tests were used..

Data management and statistical analyses were performed using SAS (version 9.4; SAS Institute, Inc.). NMF was performed using R's NMF package [49].

Results:

Characteristics of the participants

Sociodemographic characteristics of the studied participants are presented in **Table 1**. A total of 13,149 postmenopausal women were included in the analyses; 169 postmenopausal breast cancer were diagnosed during the follow-up (mean ± SD: 4.35 ±1.06 years; median: 4.83 years). Mean age at baseline was 60.5 years (SD=7.39). The majority of individuals had a graduate educational attainment, was retired and lived in more than 200,000 inhabitant urban units and were never smokers for 49% of them. One third of the sample was overweight (BMI>25 kg/m²). Most frequent physical activity levels were 'high' and 'moderate'. Overall, no significant differences on sociodemographic characteristics were found between cases and non-cases. The nutritional characteristics of the cases and non-cases are presented in**Supplementary Table S1.** Overall, no differences were observed between cases and non-cases except for organic food proportion in the diet.

The absolute estimated dietary pesticide exposure for cases and non-cases is presented inTable 2**.** Among others, the pesticide exhibiting the highest means for exposures in cases and non-cases were boscalid, iprodione, spinosad, thiabendazole, and imazalil.

The correlations between the 4 NMF-extracted components and pesticide exposure are shown **inTable 3**. Pesticides such as chlorpyriphos, imazalil, malathion, profenofos, thiabendazole were highly correlated with NMF Component 1. For NMF Component 2, highly correlated pesticides were azoxystrobin, boscalid, cyprodinil, difenoconazole, fenhexamid, iprodione, tebuconazole, lambda cyhalothrin.

NMF Component 3 was characterized by low correlations with synthetic pesticides and high correlation with organic pesticide spinosad. For NMF Component 4, high correlations with acetamiprid, carbendazim, chlorpyriphos, cypermethrin, dimethoate/omethoate were observed.

Each NMF Component exhibited specific correlates. For information, profiles and dietary

patterns are presented in **Supplementary Tables S2 to S9: Supplementary Tables S2-S3** for NMF Component 1, **S4-S5** for NMF Component 2, **S6-S7** for NMF Component 3, **S8-S9** for NMF Component 4. Main findings are the negative and positive linear associations between proportion of organic food and NMF components 1-2 and 3 respectively.

The absolute estimated dietary pesticide exposures compared across components quintiles are presented in **Supplementary Table S10** and **Supplementary Table S11**.

Correlations between dietary intakes for 33 food groups and NMF components are shown in **Supplementary Table S12.**

Associations between pesticide dietary exposure and breast cancer risk

Table 4 presents Hazard Ratios (HR) for the associations between NMF components and the risk of postmenopausal breast cancer, with several levels of adjustments. Positive and significant association was found for the fifth quintile of NMF component 1, HR=1.73, 95%CI(1.05;2.84). With regard to NMF component 3, participants in the fifth quintile had significantly lower risks (HR=0.57, 95%CI(0.34;0.93), p trend=0.006) of postmenopausal breast cancer than the first quintile ($p<0.05$). HR for the fifth quintiles of NMF Components 2 and 4 were HR 0.96, 95% CI(0.59;1.56), p-trend : 0.30 and HR 0.65, 95% CI(0.38;1.12), p-trend : 0.13.

Further adjustments for the quality of the diet (with the sPNNS-GS2 score, Model 2), and residing in an agricultural area (Model 3) did not modify the findings (**Table 4**).

Several interactions between NMF components and other variables were tested in the models (provegetarian score, sPNNS-GS2, overweight vs non-overweight). A significant interaction was found between BMI and NMF component 1 (p for interaction with BMI in 2 categories = 0.004) on the risk of postmenopausal breast cancer. Therefore, stratified analyses were performed, with a threshold of 25 kg/m², and results are shown in **Table 5**. Associations between NMF Component 1 and post-menopausal breast cancer risk were significant among

individuals with a BMI>25 kg/m² only, with higher risk for the fifth quintile and fourth quintile compared to the first quintile, $HR_{Quintile\ 5\ vs\ Quintile\ 1}$: 4.13 (95%CI(1.50;11.44) and $HR_{Quintile\ 4\ vs\ Quntile\ 1}$: 3.02 (95%CI(1.08;8.47)), p trend=0.006 (**Table 5**).

Sensitivity analyses

After exclusion of cancer cases occurring less than 1 year after baseline, HR were similar but the loss of statistical power did not allow to reach significance (**Table 6**).

Further adjustments for the percentage of ultra-processed foods, or provegetarian score, did not modify the results substantially.

Discussion:

In this large population of French postmenopausal women, we found significant negative associations between NMF component 3 (reflecting low exposure to several synthetic pesticides) and post-menopausal breast cancer risk. When analyses were stratified on BMI (threshold 25 kg/m^2), positive association between NMF Component 1 (reflecting exposure to chlorpyriphos, imazalil, malathion, thiabendazole) and postmenopausal breast cancer risk was found among overweight women. No significant associations were detected for the other components.

To our knowledge, the present study is the first to investigate various pesticide exposure patterns, accounting for farming practices in relation with breast cancer risk in the general population. Thus, our findings cannot be directly compared to previous scientific literature. However, some studies have been conducted to investigate associations between occupational, residential or domestic pesticide exposure and breast cancer risks. Studies largely focused on organochlorine (OC) pesticides and related metabolites (for instance Dichlorodiphenyltrichloroethane (DDT), Dichlorodiphenyldichloroethylene (DDE), β-Hexachlorocyclohexane (β-HCH), Hexachlorobenzene (HCB), Pentachlorothioanisole (PCTA), now banned in European Union, reporting higher breast cancer risk for users (personal or occupational) [50–52].

Breast cancer risks were found higher with exposure to OC (use vs never use) in a study published by Engel et al (2005) conducted in farmer's wives population [21]. In this study, breast cancer risks also appeared elevated regarding organophosphorus pesticide family (OP) as a whole. When analysis was performed on compounds taken separately, association was significant only for malathion. Stratification on menopausal status was performed and showed higher risks among postmenopausal women whose husbands used OC and also OP such as chlorpyrifos, diazinon and malathion. In our study, NMF component 1, positively correlated with malathion and chlorpyriphos (respective correlation coefficients 0.76 and 0.73), was

significantly associated with breast cancer risk for participants with a BMI > 25 kg/m^2. Moreover, in analyses conducted in 2015 by Lerro *et al.* in the Agricultural Health Study [22], spouses whose husband used OPs had higher breast cancer risk compared to spouses whose husbands never used OPs (RR= 1.20, 95%CI(1.01; 1.43)). However, in that study, when considering pesticides molecules separately (malathion, chlorpyriphos, terbufos), associations with breast cancer risk were no longer significant, except for chlorpyriphos, and especially for Estrogen Receptor Negative/Progesterone Receptor Negative (ER-/PR-) breast cancer risk. These observations could be interpreted in light of some kind of synergistic effects evidenced in toxicological studies when exposed to pesticide residue mixtures [6,53,54]. In the same study by Lerro et al., after stratification on menopausal status, significant association between higher breast cancer risks and use of any OPs was observed among the postmenopausal women. Again, no significant associations between OP pesticides taken separately and breast cancer risk were found. Another recent study also found elevated risks in women exposed to chlorpyrifos compared with those not exposed (OR = 3.22; 95%CI(1.38,7.53) [55]. These results are consistent with our results suggesting an association between NMF component 1 (reflecting exposure to chlorpyriphos, imazalil, malathion, thiabendazole) and postmenopausal breast cancer risk.

It is important to note that after exclusion of 'early cases' (<1 year after baseline), an important drop was observed in the $HR_{\text{Quintile 5 vs Quntile 1}}$ for Component 1 from 1.73 to 1.37. It is possible that excluded cases exhibited very specific nutritional and health characteristics linked to their imminent diagnosis and probable health deterioration linked to it. We should also note that Quintile 5 lost more cases than other quintiles (42 cases to 31) and this could somehow influence the analysis.

In our study, possible hypotheses to explain the negative associations between NMF Component 3 and postmenopausal breast cancer risk rely on the fact that besides being highly

correlated with some pesticides used in organic farming (i.e. natural pyrethrins, spinosad), this component is also negatively correlated with several synthetic pesticides (azoxystrobin, chlorpropham, methamidophos). Participants with high component 3 score, seemed generally less exposed to the synthetic studied pesticides but also less exposed to pesticides with high suspected toxicity such as chlorpyriphos, imazalil, malathion. These results are consistent with those of another study, conducted in the NutriNet-Santé cohort in 2018, that reported a negative association between high organic food score and postmenopausal breast cancer risk ($HR_{Quintile4}$= 0.66; 95% CI(0.45-0.96)) [56]. One formulated hypothesis for this association was that organic farming regulations lead to a lower frequency or an absence of pesticide residues in organic foods compared with conventional foods [25]. Thus, our present results are consistent with this hypothesis. Moreover, effects had comparable magnitude.

Mechanisms underlying these associations could be related to carcinogenic properties of some organophosphate pesticides provoking DNA damage, cell apoptosis dysregulation, epigenetic changes [57], cell signaling disruption [58], nuclear receptor binding (Aromatic hydrocarbon Receptor, AhR) [59] or oxidative stress induction [4,8,9]. It can be noted that IARC classified some organophosphate pesticides as "probably carcinogenic to humans" (Group 2A) and "possibly carcinogenic to humans" (Group 2B) [10]. Endocrine disruption potential of pesticides has also been described in toxicological studies and recently in a review by Yang et al. [7,60], and could be particularly involved in hormone-dependent breast carcinogenesis, as some pesticides are known to mimic estrogen functions [57,61]. Indeed, azole fungicides, including imazalil, for which we found high correlations with NMF Component 1, have been related to inhibition of estrogen biosynthesis in some studies [62]. These pesticides are also known to affect mitochondrial activity and oxidoreduction status [63].

When considering stratified analyses on BMI (threshold 25 kg/m^2), a positive association between component 1 and post-menopausal breast cancer risk was observed in overweight

individuals.

Several studies have found positive associations between body fat and OC pesticide blood levels, overweight subjects having higher blood levels of these pesticides [64,65]. However, it is unlikely this would be the case for OPs, which are not accumulated in adipose tissue.It is possible that there is cumulative effect between obesity and pesticide exposure on cancer risks. The specific association in overweight women could also be explained by differences in paraoxonase 1 (PON1) activity, as this enzyme is involved in lipid metabolism, but also participates in hydrolysis of organophosphate compounds [66]. Indeed, some studies have shown lower levels of PON1 activity in overweight and obese patients [67,68]. In consequence, toxicity of these pesticides could be higher for this subgroup.

More data are needed on OP pesticides, in order to fully understand underlying mechanisms of this association and potential modifying effects of BMI on breast cancer.

It should be noted that associations between NMF Components 2 and 4 and postmenopausal breast cancer risk were not statistically significant in our study. Given the lack of evidence on specific pesticide mixtures in relation to human health, it is difficult to know whether this could be due to specific non-carcinogenic patterns of the studied pesticides or to the fact that the population may not be exposed enough to experience deleterious health effects.

Limitations and strengths

Some limitations of this study should be mentioned. Firstly, the NutriNet-Santé cohort is composed of volunteers, mostly highly educated, who can be more interested in their health and dietary intakes than the general French population [69]. This implies cautions when generalizing our results to other populations.

It is important to mention that dietary intakes were self-reported through a food frequency questionnaire (FFQ) and this may have caused overestimation of organic food consumption.

Other limitations come from the database used to estimate dietary pesticide exposures, since

data were not available for animal products and the database did not contain measures for copper or sulfur-based products, widely used in organic farming, but not known as carcinogenic compounds. Measures were performed in Germany, but products from all over European Union were tested.

Another limitation that should be mentioned was that the dietary pesticide exposure was estimated and is therefore not as accurate as measuring biomarkers. It is to note that biomarkers can rarely be measured on very large samples given feasibility and cost constraints. Another disadvantage of using biomarkers is that it would not give precision on the active substances to which individuals are exposed since biomarkers are not specific to one molecule.

We should acknowledge that pesticide exposure have probably been overestimated as potential concentration or dilution effects during washing, cooking or peeling on pesticide residue levels were not accounted for [37].Finally, for this study, follow-up duration was short and the number of cases limited, given a high estimated latency period for this type of disease. This can be a limitation for causal inference and statistical power of the analyses. However, we hypothesize that dietary habits change marginally over time, therefore dietary habits four years before diagnosis were probably very similar to those 10 years before. In the same way, patterns of pesticide use in France were similar during this period, as there were changes in authorizations only for three selected pesticides (anthraquinone, methamidophos and profenofos) [70]. Nevertheless, it will be interesting to reassess cancer risks after several years, in order estimate long-term effects.

Some strengths of this study can also be put forward.

Cox regression models were adjusted for a wide range of covariates, including major confounders such as diet quality indicators. Despite a limited number of cases, the sample size

405 still allowed us to perform some stratification and sensitivity analyses in order to deepen the
406 understanding of these results and reduce confounding bias.
407

Conclusion

We observed a negative association between low synthetic pesticide exposure profile (through NMF component 3) and post-menopausal breast cancer risk. Positive association between component 1 (highly correlated to chlorpyriphos, imazalil, malathion, thiabendazole) and postmenopausal breast cancer risk was also found specifically among overweight and obese women. If confirmed by other studies, some pesticides profiles may constitute risk factors among subgroups such as those with overweight. Observed associations should be investigated in other prospective studies, in different settings, coupled with experimental studies to complement these observational studies in order to validate estimated dietary pesticide exposure. A better understanding of the impact of dietary pesticides on human health could unlock prevention strategies for the whole population through regulation.

Ethics approval :

The NutriNet-Santé study is conducted in accordance with the Declaration of Helsinki, and all procedures were approved by the Institutional Review Board of the French Institute for Health and Medical Research (IRB Inserm 0000388FWA00005831) and the Commission Nationale de l'Informatique et des Libertés (CNIL 908,450 and 909,216). All participants provided their informed consent with an electronic signature, and this study is registered in ClinicalTrials.gov (NCT03335644).

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Data availability

Data of the study are protected under the protection of health data regulation set by the French National Commission for Information Technology and Liberties (Commission Nationale de l'Informatique et des Libertés, CNIL). The data are available upon reasonable request to the study's operational manager, Nathalie Pecollo (n.pecollo@eren.smbh.univ-paris13.fr), for review by the steering committee of the NutriNet-Santé study.

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Author contributions

RV, DL, JB, SH and EK-G conducted the research.

PR performed statistical analyses and drafted the manuscript.

All authors critically helped in the interpretation of results, revised the manuscript and provided relevant intellectual input. They all read and approved the final manuscript. EK-G supervised the study, had primary responsibility for the final content, she is the guarantor.

Conflict of Interest:

None declared.

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Tables and Figures:

Table 1: Characteristics of the participants, NutriNet-Santé Study, 2014 (N=13,149)

	All participants	Non-cases	Cases	P[1]
N	13,149	12,980	169	
Age, mean (SD)	60.49 (7.39)	60.48 (7.40)	61.15 (6.43)	0.24
Monthly income per household unit, %				0.27
<€1200	9.29	9.30	8.88	
€1200-1800	21.63	21.69	16.57	
€1800-2700	28.09	28.11	26.04	
>€2700	33.61	33.54	38.46	
Unwilling to answer	7.38	7.35	10.06	
Educational level, %				0.17
Less than high-school diploma	26.73	26.81	20.71	
High school diploma	17.58	17.53	20.71	
Post Graduate	55.69	55.65	58.58	
Occupational status, %				0.26
Employee, manual worker	12.29	12.36	7.10	
Intermediate profession	12.91	12.90	13.61	
Managerial staff, intellectual	14.15	14.18	11.83	
Retired	48.92	48.82	56.21	
Self-employed, farmer	1.59	1.59	1.18	
Unemployed or never employed	10.14	10.14	10.06	
Place of residence, %				0.85
Rural community	22.98	23.00	21.30	
Urban unit with a population <20,000 inhabitants	15.77	15.76	16.57	
Urban unit with a population between 20,000 and 200,000	19.45	19.48	17.75	
Urban unit with a population >200,000 inhabitants	41.80	41.76	44.38	
Smoking habits, %				0.20
current smoker	9.00	9.04	5.92	
former smoker	42.29	42.33	39.64	
never smoker	48.71	48.64	54.44	
Body Mass Index (kg/m^2), mean (SD)	24.22 (4.64)	24.22 (4.64)	24.57 (4.45)	0.16
Body Mass Index > $25kg/m^2$, %	34.19	34.17	35.50	0.72
Physical activity, %				0.12
High	35.49	35.57	29.59	
Moderate	36.06	36.05	37.28	
Low	17.07	17.08	16.57	
Missing data	11.37	11.30	16.57	
Use of hormonal treatment for menopause, %				0.04
Yes	10.92	10.87	14.20	
No	83.30	83.29	84.02	
Missing data	5.78	5.83	1.78	
Parity, %				0.33
No children	14.47	14.43	17.75	
One child	17.56	17.59	15.38	
2 children	40.12	40.18	35.50	
More than 2 children	27.84	27.80	31.36	
Family history of cancer, %	52.25	52.19	57.40	0.18

[1]P-values for comparisons between cases and non-cases using Chi-square tests or Wilcoxon tests, as appropriate.

Table 2: Estimated pesticide exposure for cases and non-cases, lower-bound scenario, NutriNet-Santé Study, 2014 (N=13,149)

	Non-cases N=12,980		Cases N=169	
Variable	**Mean**	**SD**	**Mean**	**SD**
Acetamiprid	0.0598	0.0778	0.0548	0.0966
Anthraquinone	0.0005	0.0015	0.0005	0.0010
Azadirachtin	0.0004	0.0005	0.0003	0.0004
Azoxystrobin	0.0451	0.0453	0.0457	0.0520
Boscalid	0.1312	0.1114	0.1218	0.1096
Carbendazim	0.0564	0.0581	0.0535	0.0708
Chlorpropham	0.0607	0.0616	0.0674	0.0632
Chlorpyrifos	0.0753	0.0663	0.0758	0.0751
Cypermethrin	0.0881	0.1107	0.0815	0.1370
Cyprodinil	0.0821	0.0892	0.0782	0.0790
Difenoconazole	0.0190	0.0177	0.0175	0.0157
Dimethoate Ometoate	0.0106	0.0134	0.0090	0.0138
Fenhexamid	0.1067	0.1455	0.0897	0.1060
Glyphosate	0.0035	0.0048	0.0040	0.0057
Lambda-Cyhalothrin	0.0116	0.0112	0.0117	0.0122
Imazalil	0.8459	1.0395	0.9367	1.1376
Imidacloprid	0.0791	0.0750	0.0831	0.0750
Iprodione	0.1591	0.1833	0.1552	0.1706
Malathion	0.0003	0.0004	0.0003	0.0003
Methamidophos	0.0002	0.0003	0.0003	0.0005
Profenofos	0.0000	0.0001	0.0000	0.0001
Pyrethrins	0.0020	0.0017	0.0018	0.0015
Spinosad	0.1717	0.1870	0.1447	0.1660
Tebuconazole	0.0385	0.0471	0.0373	0.0399
Thiabendazole	0.2882	0.3247	0.3239	0.3322

Table 3: Spearman Correlations between 25 selected pesticides and NMF Components, NutriNet-Santé Study, 2014 (N=13,149)

Compounds	NMF Component 1	NMF Component 2	NMF Component 3	NMF Component 4
Acetamiprid	0.34	0.41	0.26	**0.87**
Anthraquinone	0.17	0.19	-0.06	0.18
Azadirachtin	-0.09	*-0.01	0.53	*-0.01
Azoxystrobin	0.59	**0.71**	-0.18	0.16
Boscalid	0.51	**0.90**	-0.13	0.19
Carbendazim	0.31	0.38	0.31	**0.89**
Chlorpropham	0.35	0.53	-0.30	0.12
Chlorpyrifos	**0.73**	0.44	0.11	**0.60**
Cypermethrin	0.29	0.27	0.36	**0.93**
Cyprodinil	0.50	**0.91**	-0.12	0.16
Difenoconazole	0.52	**0.68**	*0.02	0.47
Dimethoate Ometoate	0.36	0.42	0.26	**0.79**
Fenhexamid	0.46	**0.79**	-0.11	0.12
Glyphosate	0.38	0.45	-0.12	0.17
Imazalil	**1.00**	0.37	-0.11	0.14
Imidacloprid	0.51	0.24	0.20	0.56
Iprodione	0.52	**0.91**	-0.10	0.15
Lambda-Cyhalothrin	0.56	**0.84**	-0.08	0.24
Malathion	**0.76**	0.49	-0.10	0.17
Methamidophos	0.32	0.35	-0.19	0.17
Profenofos	**0.95**	0.38	-0.12	0.17
Pyrethrins	0.04	0.02	0.30	0.03
Spinosad	-0.07	-0.09	**0.99**	0.35
Tebuconazole	0.55	**0.84**	-0.10	0.19
Thiabendazole	**0.98**	0.36	-0.11	0.16

*p-value for Spearman correlation >0.05

NMF: Non-negative Matrix Factorization

Bold values denote correlation coefficients >0.60.

Table 4: Cox models for associations between dietary pesticide exposure and postmenopausal breast cancer risk, NutriNet-Santé Study, 2014 (N=13,149)

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Total	P value for trend
NMF Component 1							
Number of participants	2629	2630	2630	2630	2630	13,149	
Incident Cases	27	36	34	30	42	169	
Person-years	11,385.52	11,507.50	11,566.09	11,477.81	11,266.78	57,203.70	
Model 1[1], HR (95% CI)	1	1.34 (0.81; 2.21)	1.24 (0.75; 2.06)	1.14 (0.68; 1.93)	1.73 (1.05; 2.84)		0.09
Model 2[2], HR (95% CI)	1	1.33 (0.80; 2.19)	1.25 (0.75; 2.07)	1.15 (0.68; 1.95)	1.78 (1.08; 2.93)		0.07
Model 3[3], HR (95% CI)	1	1.32 (0.80; 2.18)	1.24 (0.75; 2.06)	1.15 (0.68; 1.94)	1.77 (1.07; 2.91)		0.08
NMF Component 2							
Number of participants	2629	2630	2630	2630	2630	13,149	
Incident cases	38	43	28	26	34	169	
Person-years	11,504.90	11,453.11	11,459.27	11,499.86	11,286.57	57,203.70	
Model 1[1], HR (95% CI)	1	1.11 (0.72; 1.72)	0.72 (0.44; 1.17)	0.69 (0.42; 1.15)	0.96 (0.59; 1.56)		0.30
Model 2[2], HR (95% CI)	1	1.09 (0.70; 1.69)	0.71 (0.44; 1.16)	0.70 (0.42; 1.16)	1.00 (0.61; 1.63)		0.38
Model 3[3], HR (95% CI)	1	1.08 (0.70; 1.68)	0.71 (0.43; 1.16)	0.69 (0.42; 1.15)	0.99 (0.61; 1.62)		0.37
NMF Component 3							
Number of participants	2629	2630	2630	2630	2630	13,149	
Incident cases	47	42	23	31	26	169	
Person-years	11,304.65	11,374.78	11,437.14	11,558.22	11,528.91	57,203.70	
Model 1[1], HR (95% CI)	1	0.88 (0.58; 1.34)	0.47 (0.29; 0.78)	0.64 (0.40; 1.01)	0.57 (0.34; 0.93)		**0.006**
Model 2[2], HR (95% CI)	1	0.89 (0.59; 1.35)	0.48 (0.29; 0.80	0.66 (0.41; 1.04)	0.59 (0.36; 0.97)		**0.01**
Model 3[3], HR (95% CI)	1	0.89 (0.59; 1.35)	0.48 (0.29; 0.80)	0.66 (0.41; 1.04)	0.59 (0.36; 0.98)		**0.01**
NMF Component 4							
Number of participants	2629	2630	2630	2630	2630	13,149	
Incident cases	36	41	33	36	23	169	
Person-years	11,294.95	11,425.20	11,459.16	11,442.46	11,581.94	57,203.70	
Model 1[1], HR (95% CI)	1	1.14 (0.72; 1.78)	0.93 (0.58; 1.50)	1.01 (0.64; 1.62)	0.65 (0.38; 1.12)		0.13
Model 2[2], HR (95% CI)	1	1.15 (0.73; 1.80)	0.95 (0.59; 1.53)	1.02 (0.64; 1.63)	0.66 (0.39; 1.12)		0.13
Model 3[3], HR (95% CI)	1	1.15 (0.73; 1.80)	0.95 (0.59; 1.53)	1.02 (0.64; 1.62)	0.66 (0.39; 1.12)		0.13

Abbreviations: NMF:Non-negative Matrix Factorization; HR: Hazard Ratio; 95% CI : 95% Confidence Interval; sPNNS-GS2: Simplified Programme National Nutrition Santé Guideline Score 2

[1]Adjusted for smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, Body Mass Index, height, family history of cancer, menopausal treatment, parity

[2]Adjusted for Model 1 + sPNNS-GS2 score

[3]Adjusted for Model 1 + sPNNS-GS2 score + residing currently in an agricultural area

Table 5: Cox models for associations between dietary pesticide exposure and postmenopausal breast cancer risk, stratified analyses on BMI, NutriNet-Santé Study, 2014 (N=13,149)[1]

	BMI <25kg/m² N=8654, 109 cases						**BMI >25kg/m²** N= 4495, 60 cases					
	Quintile 1	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**		**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	
N	**1731**	**1732**	**1732**	**1732**	**1732**		**901**	**901**	**901**	**901**	**901**	
	HR	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**P-value for trend**	**HR**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**P-value for trend**
NMF Component 1	1	1.05 (0.60; 1.85)	0.65 (0.34; 1.23)	1.02 (0.57; 1.81)	0.98 (0.53; 1.81)	0.88	1	2.83 (0.83; 6.87)	2.58 (0.90; 7.38)	3.02 (1.08; 8.47)	4.13 (1.50; 11.44)	**0.006**

Abbreviations: NMF: Non-negative Matrix Factorization; HR: Hazard Ratio; 95% CI: 95% Confidence Interval; BMI: Body Mass Index; sPNNS-GS2: Simplified Programme National Nutrition Santé Guideline Score 2

[1]All models adjusted for sPNNS-GS2 score, smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, BMI, height, family history of cancer, menopausal treatment, parity.

Table 6: Sensitivity analyses for associations between dietary pesticide exposure and postmenopausal cancer risk, NutriNet-Santé Study, 2014 (N=13,149)

Model excluding early cases (1 year)	**Quintile 1**	**Quintile 2**		**Quintile 3**		**Quintile 4**		**Quintile 5**		
N=13,120; 140 cases	**HR**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**P-value for trend**
NMF Component 1	1	1.11	(0.65; 1.90)	1.19	(0.70; 2.03)	1.02	(0.58; 1.78)	1.37	(0.80; 2.36)	0.38
NMF Component 2	1	0.96	(0.60; 1.55)	0.65	(0.38; 1.11)	0.66	(0.38; 1.14)	0.86	(0.50; 1.47)	0.23
NMF Component 3	1	0.96	(0.61; 1.50)	0.46	(0.26; 0.81)	0.62	(0.37; 1.04)	0.62	(0.36; 1.07)	**0.016**
NMF Component 4	1	1.22	(0.75; 2.01)	1.15	(0.69; 1.91)	1.00	(0.59; 1.69)	0.59	(0.32; 1.10)	0.10
Model with additional adjustment for ultra-processed foods[2]	**Quintile 1** N=2629	**Quintile 2** N=2630		**Quintile 3** N=2630		**Quintile 4** N=2630		**Quintile 5** N=2630		
N=13,149; 169 cases	**HR**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**P-value for trend**
NMF Component 1	1	1.33	(0.80; 2.19)	1.24	(0.75; 2.07)	1.15	(0.68; 1.94)	1.77	(1.08; 2.92)	0.08
NMF Component 2	1	1.09	(0.70; 1.69)	0.71	(0.43; 1.16)	0.70	(0.42; 1.16)	0.99	(0.61; 1.62)	0.37
NMF Component 3	1	0.88	(0.58; 1.34)	0.47	(0.29; 0.79)	0.64	(0.40; 1.03)	0.58	(0.35; 0.96)	**0.009**
NMF Component 4	1	1.15	(0.73; 1.80)	0.94	(0.59; 1.52)	1.01	(0.64; 1.62)	0.65	(0.38; 1.11)	0.12
Model with additional adjustment for provegetarian Score[3]	**Quintile 1** N=2629	**Quintile 2** N=2630		**Quintile 3** N=2630		**Quintile 4** N=2630		**Quintile 5** N=2630		
N=13,149; 169 cases	**HR**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**P-value for trend**
NMF Component 1	1	1.33	(0.80; 2.19)	1.23	(0.74; 2.04)	1.13	(0.67; 1.91)	1.72	(1.04; 2.82)	**0.009**
NMF Component 2	1	1.09	(0.70; 1.70)	0.71	(0.43; 1.16)	0.69	(0.41; 1.14)	0.96	(0.59; 1.56)	0.30
NMF Component 3	1	0.88	(0.58; 1.34)	0.47	(0.29; 0.78)	0.64	(0.40; 1.02)	0.57	(0.34; 0.95)	**0.008**
NMF Component 4	1	1.14	(0.73; 1.79)	0.94	(0.58; 1.51)	1.01	(0.64; 1.62)	0.65	(0.38; 1.12)	0.12

Abbreviations: NMF: Non-negative Matrix Factorization; HR: Hazard Ratio; 95 % CI: 95% Confidence Interval; BMI: Body Mass Index; sPNNS-GS2: Simplified Programme National Nutrition Santé Guideline Score 2

[1]Adjusted for sPNNS-GS2 score, smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, BMI, height, family history of cancer, menopausal treatment, parity

[2]Adjusted for sPNNS-GS2 score, smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, BMI, height, family history of cancer, menopausal treatment, parity and percentage of ultra-processed foods in the diet

[3]Adjusted for provegetarian score, smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, BMI, height, family history of cancer, menopausal treatment, parity