UiT The Arctic University of Norway

The Faculty of Health Sciences

Effect of PCBs in plasma on risk of postmenopausal breast cancer

With data from the Norwegian Women and Cancer Study

Tonje Koldal Opsal

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Supervisor: Charlotta Rylander

Preface

The purpose of this report was to further investigate the health effects of persistent organic pollutants on humans, by looking into the connection between PCBs and breast cancer, using a prediction model and data from the Norwegian Women and Cancer study.

The human impact on the environment is a current issue that demands attention. For many years, the handling of garbage, pesticides and dangerous materials have been and still is neglected, maybe due to lack of knowledge, maybe due to ignorance. Years later, the consequences may be catching up to us. The connection between climate, environment and human health has long been an interest of mine, and especially environmentally related cancer.

Last year I contacted my mentor, Jan Magnus Kvamme and asked him if he knew of anyone who could supervise me in a thesis about the environment and cancer. He introduced me to Guri Skeie from the Department of Community Medicine, who presented many interesting options and ideas for me to write about. When I told her of my interest in environmentally related diseases, she further introduced me to Charlotta Rylander, who had outlined a thesis about POP's and cancer risk, a subject that immediately caught my attention.

I would like to give a huge thanks to my supervisor Charlotta, or "Lotta", for patience, encouraging words and hours of guidance. Writing this thesis would not have been possible without her.

Strandvik, May 30, 2021

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Abstract

Background: Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants suspected to cause negative health effects such as cancer. However, the findings on breast cancer are inconsistent, and lack data on larger populations. The objective in this master thesis is to study the association between estimated plasma concentration of summed PCBs and the incidence of postmenopausal breast cancer overall, as well as hormonal receptor positive and negative postmenopausal breast cancer.

Methods: I used data from 48,675 participants in the population-based Norwegian Women and Cancer cohort and predicted summed PCB concentrations from a previously developed linear regression model. Participants were afterwards categorized into tertiles of low, medium, and high levels of summed PCBs. Multivariable Cox proportional hazard regression was used to assess the association between PCB exposure and incidence of postmenopausal breast cancer, including selected subtypes.

Results: I found significant associations between higher predicted plasma concentrations of summed PCBs and the incidence of postmenopausal breast cancer (Moderate vs low: HR = 1.09, 95 % CI: 0.97-1.23, high vs low: HR = 1.22, 95 % CI: 1.08-1.38, p trend < 0.01), including ER+ (Moderate vs low: HR = 1.01, 95 % CI: 0.97-1.25, high vs low: HR = 1.18, 95 % CI: 1.04-1.35, p trend = 0.013) and PR+ (Moderate vs low: HR = 1.20, 95 % CI: 0.97- 1.47, high vs low: HR = 1.27, 95 % CI: 1.02-1.57, p trend = 0.036) subtypes. No association was found when assessing ER- and PR- postmenopausal breast cancer (p_{trend} ER- = 0.627, p_{trend} PR- = 0.580).

Conclusion: Higher predicted plasma concentrations of summed PCB increase the risk of postmenopausal breast cancer, ER+ and PR+ postmenopausal breast cancer, but not ER- and PR- postmenopausal breast cancer.

Abbreviations

BMI: Body mass index

CI: Confidence Interval

DAG: Directed Acyclic Graph

ER: Estrogen receptor

HER-2: Human epidermal growth factor receptor 2

HR: Hazard ratio

IARC: International Agency for Research on Cancer

ICD-10: International Classification of Diseases, revision 10

MHT: Menopausal hormone therapy

NOWAC: The Norwegian Women and Cancer Study

OC: Oral contraceptive

PCB: Polychlorinated biphenyl

pg: picograms

PR: Progesterone receptor

POP: Persistent Organic Pollutant

SSB: Statistics Norway (Statistisk Sentralbyrå)

STATA: Software for Statistics and Data Science

WCRF: World Cancer Research Fund

+/-: positive/negative

1 Background

1.1 Persistent organic pollutants

Persistent organic pollutants (POPs) are toxic chemical substances appearing as part of pesticides and industrial products, such as electronics, insulation materials and textiles (1). They may also develop as byproducts from combustion, waste management and heating, and thereafter be discharged into the environment, where they are spread by means of natural processes such as ocean- and air currents (1;2). Due to their persistence and ability to accumulate in adipose tissues they are therefore globally distributed and eventually concentrated in our food chains, making food the most important source of exposure for humans(1;3).

In general, proving the effects of environmental contaminants on human health is difficult. We are exposed to a wide range of substances in different concentrations over a substantial period of time, which makes it challenging to attribute the effects to specific pollutants (4). Many researchers suggest that POPs may cause serious health effects, including several cancer types and disorders of the immune system(2). Some POPs have even been associated with endocrine effects, suggesting a disruption of the hormonal system which further may cause damage on the reproductive system and thereby the descendants of the exposed individuals (2;5, p. 111). Human health effects of POPs are, however, an on-going area for research, and short- and long-term effects are yet to be fully determined (6;7, p. 28).

In 2004, a global treaty was formed to protect the environment from these chemicals. The Stockholm Convention initially included a list of twelve POPs considered to cause adverse effects on humans, animals, flora, and fauna, a number that is more than doubled today (7, p. 9). Among the substances included on the list since the beginning, we find polychlorinated biphenyls (PCBs) (7, p. 29).

1.1.1 PCBs

PCBs are well known POPs and consist of 209 different congeners with high lipophilicity and a great storage capacity in adipose tissue (8). Since their introduction in the 1930s, PCBs have been utilized in buildings, electrical equipment, sealant and painting, among many other things (8). Like other POPs, they eventually spread globally and accumulate in our food chains. In fact, according to The Norwegian Institute of Public Health, over 90 percent of humans exposure to PCBs today happens through the diet, mainly via fatty fish, such as herring,

mackerel, halibut, salmon, and trout (8;9, p. 15). However, levels of PCBs in human plasma are not only dependent on exposure, but also factors like weight change, body mass index (BMI), birth year, breastfeeding, and parity (5, p. 249;10;11).

When humans are exposed to PCBs over time, the accumulation in liver and fatty tissue may lead to several negative health effects. Important findings suggest that PCB exposure can cause cancer and damage to the immune system (6). For instance, World Cancer Research Fund (WCRF) posted a report in 2019, informing that PCBs are strongly associated with an increased risk of skin cancer (12, p. 14). In addition to these effects, studies have found that when a pregnant woman is exposed, the substances can be transferred to, and possibly injure the fetus (9, p. 15).

PCBs' toxic characteristics eventually led to restrictions in the industry and since 1980 the substances have been forbidden to use and sell in Norway (8;13). However, due to their persistence, high levels of PCB can still be measured in several of our fiords and harbor basins (8). The concentration of PCBs in the Norwegian diet is continuously monitored, and a tolerance limit is set to protect against the possible damaging effects. As late as in 2018, this limit was lowered to a significantly lower number than the previous limit from 2001 (14).

1.2 Breast cancer

Breast cancer is defined as a malignant tumor in breast tissue, primarily lobular or ductal carcinomas or carcinomas in situ (15). In 2019, 3726 women were diagnosed with breast cancer in Norway (16). It is the most common form of cancer among Norwegian women, and the most frequent form of cancer in the world (16;17). Even though we don't know the exact reason why many women get breast cancer, we do know of various risk factors such as heritage, sex, hormonal condition, atypical hyperplasia, ionizing radiation, estrogen use before the age of 35, long lasting postmenopausal estrogen therapy, previous breast cancer, overweight and alcohol (18). Average age at the time of diagnosis is 59 years, and the risk increases with age (19).

WCRF published a report in 2018, focusing on how diet, nutrition and physical activity affects the risks of pre- and postmenopausal breast cancer (20). Findings on postmenopausal breast cancer included strong evidence that being physically active, breastfeeding, and being overweight or obese in young adulthood decreases the risk of the disease (20, p. 11). On the other hand, being tall, being overweight, obesity, or gaining weight in/throughout adulthood increases the risk of postmenopausal breast cancer. The evidence on dietary risk factors is

limited but shows that consuming foods containing carotenoids and diets containing high levels of calcium might decrease the risk of postmenopausal breast cancer, while consuming non-starchy vegetables may be associated with a decreased risk of estrogen receptor (ER) negative breast cancer (20, p. 11).

1.2.1 Subtypes of breast cancer

In proven invasive carcinomas, all tumors are analyzed for the biomarkers HER-2, Ki-67, and hormonal receptor status using immunohistochemistry and/or in situ hybridization (21). The results decide the tumors' subtype definition, which affects both the choice of treatment and the patient's prognosis, as every subtype is different in both its natural history and in its response to treatment. About 75-85 % of breast cancer cases are hormone receptor positive, meaning that the tumor is "nurtured" by estrogen and/or progesterone (22, p. 166). The other biomarkers are HER2-status and levels of Ki-67, both saying something about the level of proliferation in the cells. The subtypes are classified using molecular gene profiles (23).

ERs are normally expressed in 10-20 % of normal breast tissue, and the normal levels of estradiol range from 15 to 200 picogram (pg) per mL in premenopausal women, to 10 to 20 pg/mL in postmenopausal women (22, p. 166;24). In healthy individuals, estrogen participates in the regulation of development and further growth of mammary glands (25). The ER has been named the paradigm tumor marker when managing cancer (24). This is because the expression of ERs in a tumor is correlated with a positive prognosis, and accordingly a negative prognosis when not expressed (22, p. 166;24). The role of PR is being researched but is yet to be fully established. However, response to endocrine treatment has been observed in patients with ER negative and PR positive breast cancer (22, p. 175).

1.3 PCB and breast cancer

The International Agency for Research on Cancer (IARC) classified PCB as a human carcinogen in 2013 (5, p. 439). This was based on findings that indicated an association between exposure to PCBs and the incidence of malignant melanoma, in addition to a positive association to both non-Hodgkin's Lymphoma and breast cancer.

One reason to investigate the effects of PCBs on breast cancer is their ability to accumulate in adipose tissue, which further suggests a possibility of accumulation in breast tissue. Several studies have included mammary biopsies to measure PCB levels in breast tissue when

investigating the connection between breast cancer and PCB exposure, however, with contradictory results (26-30).

Another reason to study the possible correlation are the suggested endocrine effects of PCBs. The substances have proven to have both estrogen stimulating and estrogen blocking effects, depending on congener and tissue (5, p. 392). A study of the connection between PCBs and breast cancer in general, as well as the relation to the different subtypes may therefore be appropriate, as the subtypes represent different hormonal impacts on the tumors (31).

1.4 Aim

The aim of this thesis is to study the association between predicted plasma concentration of summed PCBs and the incidence of postmenopausal breast cancer and subtypes in participants of The Norwegian Women and Cancer Study (NOWAC).

The conclusions from earlier studies are mainly based on results from experimental studies and cell lines (5). An epidemiologic study will therefore be able to study associations in a much larger data material.

2 Material and method

2.1 Material

This thesis is based on data from NOWAC at The Faculty of Health Sciences of the University of Tromsø (32). NOWAC is a cohort study initially created to study the association between breast cancer and the use of oral contraceptives, as well as other risk factors associated with breast cancer (33). With near 172,000 participants and data collected since 1991, the study contributes to epidemiologic cancer data able to compete on an international level (33). The participants were invited through a letter sent to their home address, and include Norwegian women aged 30 to 70 years old at time of recruitment, randomly selected from the national population register in cooperation with Statistics Norway (SSB). The external validity is evaluated and found satisfactory (32).

The participants answered questionnaires containing questions about health status, diet, medication and life style areas such as smoking, height, weight, physical activity, family history and other sociodemographic factors (32). New researchers are then able to study cancer risk in relation to different types of exposure, as well as accounting for many possible confounding factors (34). Blood samples has been included in the study since 2006, allowing further

investigation of the plasma concentration of specific substances (34). That presented the opportunity to observe connections between accumulation of PCBs and incidence of different cancer types. Numerous researches have benefitted from this addition to the study (34).

This study contains data from 63,154 women included in the study in year 2002, 2004 and 2005. The NOWAC food frequency questionnaire (FFQ) allows participants to record their consumption of more than 90 different foodstuff during the preceding year (35). It has been included in the questionnaires since 1996, however with variable level of detail. The FFQ records from 2004 were validated by Hjartåker, et. al., where 283 participants were selected for four 24 h recalls (35). They concluded that the FFQ presented satisfactory accuracy when estimating daily intake of several food items. This study also includes reported body weight from 1991, 1998, 2004 and 2005. These self-reported measurements have been validated in a smaller sample by trained health personnel and found sufficiently accurate (36).

In addition to answering questionnaires, 50,000 women have delivered a blood sample (37). Of the blood samples collected, 326 were analyzed for POPs, including PCBs. 259 of the samples were used in a study conducted by Berg, et. al. to develop linear regression models predicting plasma concentrations of PCB-153, PCB-180 and summed PCBs based on sociodemographic variables and the participants diets (10). The study compared measured plasma levels of PCB to the predicted plasma levels and concluded, based on correlations and adequate inter-method agreements, that the models achieved satisfactory precision, especially when classifying the subjects in groups based on low, medium, and high exposure.

2.2 Method

This project is a cohort study where a statistical prediction model is used to estimate the blood concentration of summed PCBs in 48,765 women participating in the NOWAC study (10). In this context, summed PCBs include the congeners PCB-118, PCB-138, PCB-153 and PCB-180.

2.2.1 Exclusions

The original data consisted of 63,154 participants, including 12,355 cancer cases (Figure 1). I excluded 3845 women with prevalent cancer at baseline, as well as three women that had emigrated or died before registration of baseline questionnaire. Implausible values on weight, weight change per year and height were excluded from the study as well(n=12). I also excluded extreme values of menarche and menopause (n=204). For this exclusion I set a lower limit of 8

years and 35 years, respectively, as these are the defined ages of precocious puberty and menopause (38;39). Lastly, I excluded women aged under 12 years or over 55 years at the time of their first full term pregnancy (n=1).

As this study aimed to assess postmenopausal breast cancer, I chose to exclude 10,324 pre- and perimenopausal women, including women under 53 years old with unknown menopausal status from the data. Following the Million Women Study (40), postmenopausal women were defined as those who naturally stopped menstruating, those not menstruating with listed age at menopause, those using hormone replacement therapy and women with a reported hysterectomy and/or ovariectomy. Women aged 53 or older with an unknown menopausal status were also included. This definition of postmenopausal women was validated by Waaseth, et. al. in 2008 (37). The final study sample included 48,765 participants, of which 2,036 were diagnosed with postmenopausal breast cancer during follow-up. Of these breast cancer cases, 1,669 were classified as ER+, 203 as ER-, 642 as PR+ and 524 as PR- tumors (Figure 1).

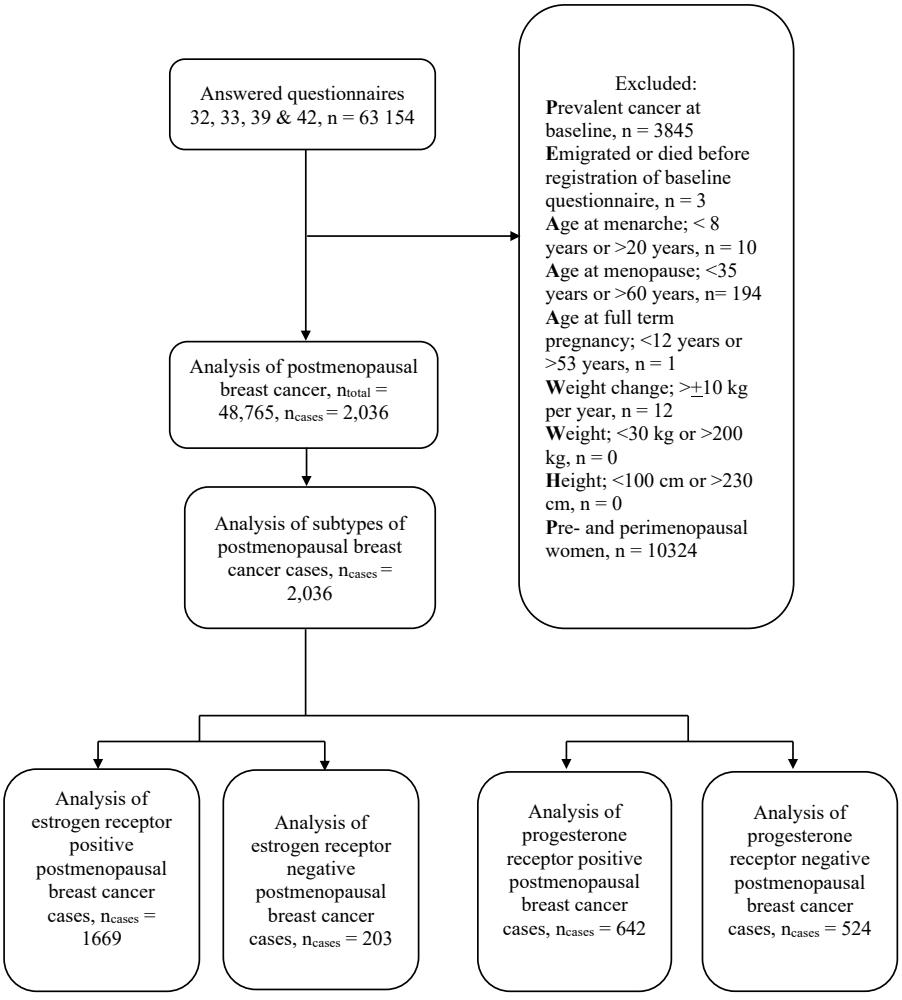

Figure 1 - Flow chart of inclusion and exclusion criteria

2.2.2 Estimation of PCB exposure

Table 1 presents the linear regression model, used to predict the plasma concentration of summed PCBs. The table is derived from the original prediction model by Berg et al(10). After calculating the predicted concentrations, the women were classified into tertiles based on low, medium, and high levels of predicted plasma concentrations of PCBs.

Table 1 – Linear regression model for predicting plasma concentrations on summed PCBs in participants of NOWAC

	Summed PCBs
Constant	678
Predictors[a]	*Regression coefficient (β-values)*[b]
Birth year	-8.20
Breastfeeding (months)	-2.05
Weight change (kg/year)	-68.7
Boiled cod (g/day)	0.84
Brown cheese (g/day)	-1.29
Fish liver (g/day)	55.90
Jam (g/day)	-1.25
Cabbage (g/day)	2.70
Pancakes (g/day)	-1.29
Spirits (g/day)	2.89
Steak (g/day)	-2.15
Vegetables, mix (g/day)	-0.81

[a] Predictors were included as continuous variables.
[b] β-values express the change in POP concentrations (ng/g lipid) per unit increase (1 g/day for the dietary variable) in the predictor (10)

2.2.3 Categorization

To simplify the analysis, the data was categorized into subgroups. I calculated the participants' BMIs from self-reported body weight (kg) and height (meters(m)) and defined them according to WHO's definition of BMI categories as under- or normal weight (<25 kg/m^2), overweight (25.0-29.9 kg/m^2) or obese (≥ 30 kg/m^2). Years of completed education were divided into three groups, where 9 years or less equals completed secondary school, high school is 10-12 years, and higher education is over 12 years. All participants completed primary- and secondary school before the reform in 1997 where mandatory education was expanded from 9 to 10 years (41).

The number of completed pregnancies was categorized as no children, 1-2 children or 3 or more children and then combined with age at first full term pregnancy, creating five new variables:

nullipara, <30 years; unipara, ≥ 30 years; unipara, <30 years; multipara, and ≥30 years; multipara. The menopausal status was divided into categories premenopausal, perimenopausal, postmenopausal and unknown, following the criteria as mentioned under "2.2.1 Exclusions". I defined the use of menopausal hormone therapy (MHT) and use of oral contraceptives (OCs) as never, former, or current user. Smoking was categorized in the same manner, as never, former, or current smoker. The participants recorded physical activity on a 10-point scale, which I divided into four levels: No/minimal (1-4), moderate (5-6) and high (≥ 7) level of physical activity.

I identified women diagnosed with malignant neoplasm of the breast (ICD-10: C50) and classified the subtypes according to the Norwegian guidelines (21). The Norwegian Breast Cancer Group published a change in ER classification in 2010, where the threshold for classifying a tumor as ER negative (ER-) changed from < 10 % reactivity to < 1 % reactivity (42). Accordingly, a tumor classified as ER positive (ER+) displayed ≥ 10 % reactivity prior to 2010, and ≥ 1 % after. In an e-mail sent May 2021 by O. M. Mangrud, chief physician at The Norwegian Cancer Registry (Ok.Malfrid.Mangrud@kreftregisteret.no), she states that it is impossible to know exactly when the new recommendations were followed by the different pathology departments in Norway, but its assumable that they all changed the clinical practice during 2010. This study will therefore apply the old definitions (ER- <10 %, ER+ ≥10%) before January 2011 and the new definitions (ER- <1 %, ER+ ≥1%) after.

Results will be presented with mean, SD and with percentage for grouped variables.

2.2.4 Statistical analysis

The association between predicted plasma concentrations of summed PCBs and the incidence of postmenopausal breast cancer was investigated using Cox proportional hazard regression. Entry time was date of enrolment, and exit time was date at cancer diagnosis, death, emigration, or end of follow-up, whichever occurred first. Failure was set as breast cancer diagnosis. The lowest tertile of predicted plasma concentration of summed PCBs was used as reference group in every analysis.

Cox proportional hazard regression was also used to assess the subtype specific postmenopausal breast cancer risk, looking into ER+, ER-, PR+ and PR- breast cancer. To test for linear trend (p_{trend}), the group identifier was replaced with the median of predicted plasma concentration of

summed PCB per tertile and included in the multivariable model. Schoenfeld's residuals were used to assess the proportional hazards assumption.

Confounding factors were identified using a directed acyclic graph (DAG), created in the DAGitty application (43) (Figure 2). Initially the model also included age, breastfeeding and weight change, as these are factors affecting both risk of postmenopausal breast cancer and plasma levels of PCBs (10;20, p. 11). However, as these variables were included in the prediction model (10), I did not include them in the multivariable adjusted analysis. Based on the calculated suggestions from the DAGitty application, the variables included in the multivariable adjusted analysis were BMI, education and use of oral contraception. The multivariable adjusted analysis was conducted as a complete case analysis, where the individuals with missing information on any of the included variables were excluded from the regression model.

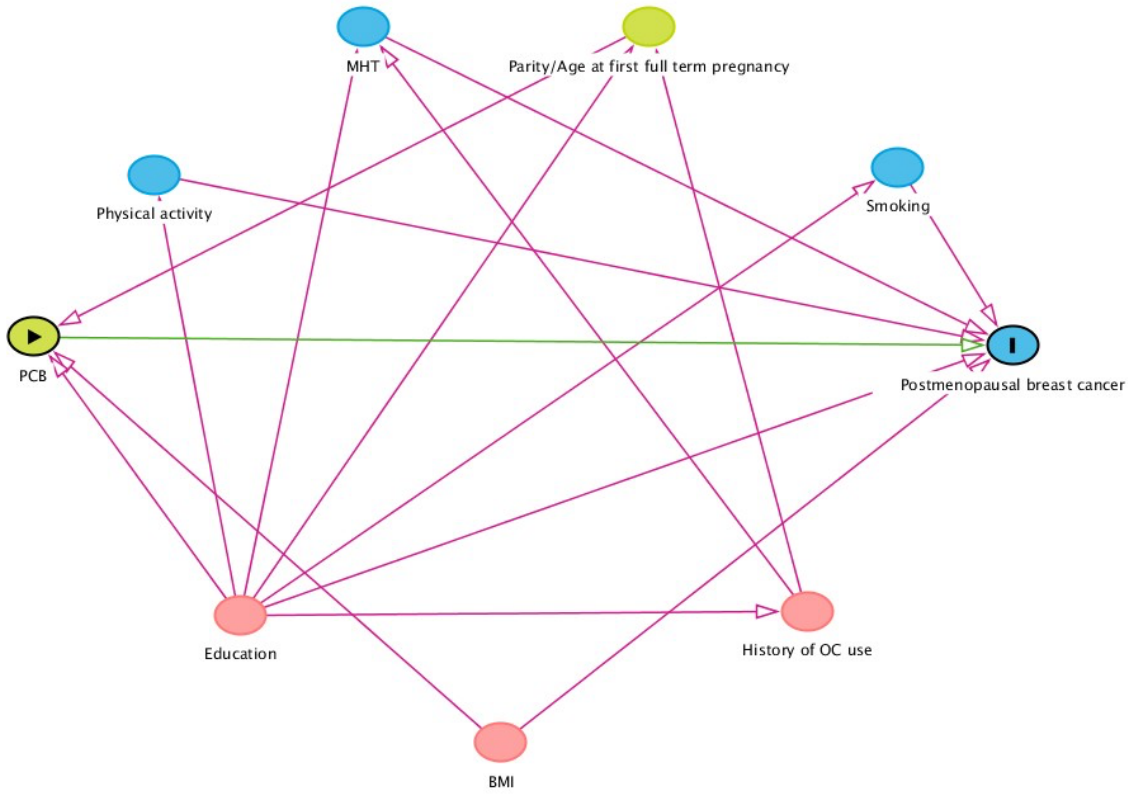

Figure 2 - Causal Diagram of PCB and Postmenopausal breast cancer

To assess the level of significance I used a P-value of 5 %. The statistical analysis was conducted using Stata, version 16.1 (44).

3 Results

3.1 Characteristics

After exclusions, the study sample included a total of 48,765 women. 2,036 of these women were diagnosed with postmenopausal breast cancer during the follow-up period. The mean (standard deviation, SD) age at enrolment was 58.4 (6.2) years. The participants were classified into three equal sized groups based on their predicted concentration of summed PCBs: 15,080 participants in group 1 (low), and 15,079 participants in both group 2 (medium) and 3 (high). The mean (SD) predicted concentration of summed PCBs in group 1, was 137.9 (50.1) ng/g, in group 2; 234.6 (22.5) ng/g and in group 3; 353.9 (66.8) ng/g.

Compared to women with low predicted concentrations of summed PCBs, women in the highest tertile of summed PCBs were older, less educated, had lower prevalence of overweight/obesity, had more often 3 children or more and were more physically active. Most of the women in the highest tertile had never used oral contraceptives, as opposed to the lowest tertile, where most women were previous users. No considerable difference between the groups was seen in age at menopause, age at menarche, use of menopausal hormone therapy, smoking or age at first completed pregnancy.

Table 2 – Study sample characteristics according to tertiles of predicted plasma values of summed PCBs

	Predicted plasma concentration of summed PCBs		
Characteristics	**Low**	**Moderate**	**High**
n	15080	15079	15079
Summed PCBs (mean [SD])	137.9 (50.1)	234.6 (22.5)	353.9 (66.8)
Incidence of postmenopausal breast cancer	610	639	631
Estrogen receptor status (%)			
ER+	89.0	89.3	89.1
ER-	11.0	10.7	10.9
Progesterone receptor status (%)			
PR+	52.1	55.4	58.1
PR-	47.9	44.6	41.9
Age at baseline (mean [SD])	54.3 (4.0)	57.5 (4.2)	63.2 (6.2)
School years (%)			
<10 years	22.0	27.6	41.6
10-12 years	36.2	35.9	31.4
>12 years	41.8	36.5	27.0
BMI (%)			
Normal-/underweight	44.8	55.6	55.8
Overweight	38.3	34.6	34.2

Obese	16.9	9.8	10.0
Age at menopause (mean [SD])	48.8 (4.7)	49.6 (4.8)	49.5 (5.4)
Age at menarche (mean [SD])	13.3 (1.4)	13.4 (1.4)	13.5 (1.4)
Age at first completed pregnancy (mean [SD])	23.9 (4.2)	23.7 (4.1)	23.7 (4.1)
Parity (%)			
No children	5.5	8.1	9.1
1-2 children	50.8	54.7	44.9
3 or more children	43.7	37.2	46.0
Use of oral contraception (%)			
Never	39.8	45.4	64.1
Previous	60.0	54.5	35.8
Current	0.2	0.1	0.1
Use of menopausal hormone therapy (%)			
Never	49.8	42.0	48.7
Previous	25.3	30.3	26.2
Current	24.9	27.7	25.1
Smoking			
Never	38.1	37.0	38.9
Previous	33.1	33.8	32.6
Current	28.8	29.2	28.5
Level of physical activity			
Low	24.8	22.2	24.9
Moderate	38.3	38.2	33.5
High	36.9	39.6	41.6

3.2 PCB and postmenopausal breast cancer

Participants with elevated predicted concentrations of summed PCBs experienced an increased incidence of postmenopausal breast cancer (moderate vs low: HR = 1.09, 95 % CI: 0.97-1.23, high vs low: HR = 1.22, 95 % CI: 1.08-1.38). These findings suggest that higher levels of PCB are associated with an increased risk of breast cancer in a linear dose response manner (p trend= <0.01) (Table 3). Similar results occurred when observing the associations between predicted concentrations of summed PCBs and, ER + (moderate vs low: HR = 1.01, 95 % CI: 0.97-1.25, high vs low: HR = 1.18, 95 % CI: 1.04-1.35, p trend = 0.013) and PR+ (Moderate vs low: HR = 1.20, 95 % CI: 0.97- 1.47, high vs low: HR = 1.27, 95 % CI: 1.02-1.57, p trend = 0.036) postmenopausal breast cancer. No association was observed between predicted concentrations of summed PCBs and ER- or PR- postmenopausal breast cancer (p_{trend} ER- = 0.627, p_{trend} PR- = 0.580).

As seen in table 3, the Multivariable HR 95 % CI crosses 1 when comparing moderate levels of predicted plasma summed PCBs with low levels, in every conducted analysis. This implies that the results in the mentioned categories are not statistically significant, even though the value of p_{trend} is under 5 %. The model assumption of Schoenfeld's residuals was met.

Table 3 – Hazard ratios (HRs) and 95 % confidence intervals (CIs) for associations between predicted concentrations of summed PCBs and postmenopausal breast cancer cases

	Univariate analysis			Multivariate analysis			
Tertiles of predicted plasma concentration of summed PCBs	**n**	**Postmenopausal breast cancer cases (n_{cases})**	**HR (95 % CI)**	**n**	**Postmenopausal breast cancer cases (n_{cases})**	**Multivariable HR (95% CI)**	**P (trend)**
Low	15080	610	1	14455	584	1	<0,01
Moderate	15079	639	1.07 (0.96, 1.19)	14261	604	1.09 (0.97, 1.23)	
High	15079	631	1.13 (1.01, 1.26)	13310	568	1.22 (1.08, 1.38)	
		Estrogen receptor positive (ER+) postmenopausal breast cancer cases			Estrogen receptor positive (ER+) postmenopausal breast cancer cases		
Low		511	1		489	1	0.013
Moderate		535	1.07 (0.95, 1.21)		506	1.10 (0.97, 1.25)	
High		503	1.08 (0.95, 1.22)		456	1.18 (1.04, 1.35)	
		Estrogen receptor negative (ER-) postmenopausal breast cancer cases			Estrogen receptor negative (ER-) postmenopausal breast cancer cases		
Low		62	1		61	1	0.704
Moderate		63	1.04 (0.73, 1.48)		61	1.04 (0.72, 1.48)	
High		59	1.06 (0.74, 1.51)		52	1.08 (0.74, 1.58)	
		Progesterone receptor positive (PR+) postmenopausal breast cancer cases			Progesterone receptor positive (PR+) postmenopausal breast cancer cases		
Low		182	1		170	1	0.036
Moderate		209	1.16 (0.96, 1.42)		197	1.20 (0.97, 1.47)	
High		204	1.20 (0.98, 1.46)		182	1.27 (1.02, 1.57)	
		Progesterone receptor negative (PR-) postmenopausal breast cancer cases			Progesterone receptor negative (PR-) postmenopausal breast cancer cases		
Low		167	1		162	1	0.580
Moderate		168	1.03 (0.83, 1.27)		160	1.04 (0.84, 1.30)	
High		147	0.97 (0.78, 1.21)		135	1.07 (0.84, 1.35)	

⁎Multivariable adjusted for BMI, education and use of oral contraception

4 Discussion

In this study, I found evidence that higher predicted plasma concentrations of summed PCBs were associated with an increased risk of postmenopausal breast cancer. These findings also applied for the subtypes ER+ and PR+ postmenopausal cancer. As for ER- and PR-, there were no evidence of associations with predicted sum PCB concentrations.

Calaf et al. describes several mechanisms of PCBs in humans that might support my findings (45). Firstly, PCBs lipophilic and accumulative properties cause bioaccumulation in human tissues such as breast tissue. Secondly, certain PCBs have shown estrogenic or antiestrogenic effects, and may influence ER. Thirdly, and most importantly, PCBs have demonstrated well known carcinogenic characteristics such as alteration of cell proliferation, cell death or nutrient supply, genotoxicity, immunosuppressive effects, epigenic alterations and induction of oxidative stress (5, pp. 430-439;45).

To my knowledge this study is the largest prospective cohort study assessing associations between predicted plasma concentrations of summed PCBs and the incidence of postmenopausal breast cancer. In WCRFs third expert report from 2018, PCBs were listed as limited/not conclusive when investigated in relation with postmenopausal breast cancer (20, p. 11). However, as these findings were not part of the continuous update project they were not further investigated and remained the same as in the Second Expert Report, published in 2007 (20, p. 95;46).

Another systematic review with inconsistent findings was conducted in 2016 by Mouly and Toms (47). In contrast to this study, they found no evidence of association between breast cancer risk and PCB exposure. In their review, they included eight case-control studies, of which only three measured plasma concentration of PCBs before time of diagnose (47). None of the three studies found a significant association between PCBs and breast cancer.

In 2019 Ennour-Idrissi et. al. conducted a systematic review and critical appraisal of the literature concerning POPs and breast cancer (48). Among the chosen literature, plasma concentrations of PCBs were included in 38 studies. The authors concluded that PCB-118, PCB-138, PCB-170 and PCB-180 were consistently positively associated with breast cancer risk (48), thus partly agreeing with my findings.

Some studies have chosen to classify the PCBs into three groups based on their biological, structural, and pharmacokinetic properties (49;50). Group I contains potentially estrogenic PCBs, group II potentially anti-estrogenic and immunotoxic dioxin-like and group III phenobarbital, CYP1A and CYP2B inducers and biologically persistent PCBs (49). PCB-153, a congener highly correlated to summed PCBs, can be placed in Group III (50;51). A meta-analysis from 2015 found evidence that group II and III might contribute to the risk of breast cancer (49), suggesting an agreement with my results.

Several studies investigating the association between PCBs and breast cancer have reported a problem regarding time of exposure (52). The PCB plasma prediction model is based on questionnaire data reported at a certain time of life, not taking into consideration the amount of exposure before participating in the study, nor the time from exposure to cancer diagnosis. Research has demonstrated that PCBs disturb hormone levels (53) and my findings suggest a connection between PCBs and the subtypes ER+ and PR+ postmenopausal breast cancer. It may therefore be assumed that the effects of exposure may vary with different hormonal levels, for example during breast development in puberty.

The systematic reviews and meta-analysis mentioned above included case-control and cohort studies with measured levels of PCB in blood, breast adipose tissue, other adipose tissue, and breast tumors (47-50). None of them reported the use of a PCB-prediction model, as utilized in this study. The advantages of using such a model includes the possibility of predicting plasma concentration in women participating in NOWAC, without having to sample blood and conduct time consuming analysis. The analyses of PCBs are expensive, and often limits the amounts of participants in similar studies (10). The prediction model makes it possible to study a larger population, which provides several advantages. Firstly, it allows a cohort design, where a larger sample equals more statistical strength to discover a correlation between exposure and outcome. Secondly, a larger sample makes the results more representative of the general population.

On the other hand, challenges may arise when utilizing the prediction model. To predict the plasma concentration of PCBs, the variables age, breastfeeding, and weight change were used, known to affect both risk of postmenopausal breast cancer and plasma levels of PCBs (10;20, p. 11). When conducting a multivariable analysis normally one would adjust for all confounding factors, but as these three were already included in the prediction model, adjusting for them would generate incorrect exposure data. For this reason, I chose to not adjust for these variables, which could possibly affect the results.

Another possible source of error in studies like these, is the self-reported information. There is no "gold standard" reference method in reporting dietary intake. FFQ is among the best alternatives when collecting data for dietary assessment in large samples, especially when it comes to cost and time investment (54). However, even though it has been validated and considered as acceptable (35), measurement and reporting bias may cause attenuated results.

Lastly, my study observes summed PCBs, not considering the different properties of the 209 congeners. Studies have shown that some PCBs demonstrate estrogenic, and others antiestrogenic effects (5, p. 392), suggesting a possible antagonistic effect if exposed to both forms.

5 Conclusion

Results from this population-based prospective cohort study suggest that higher predicted plasma levels of summed PCB increase the risk of postmenopausal breast cancer, ER+ and PR+ postmenopausal breast cancer, but not ER- and PR- postmenopausal breast cancer. Further studies should be conducted to investigate how distinct congeners of PCBs and time of exposure to them may affect the risk of postmenopausal breast cancer.

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Appendix

Summary of key references. As the main part of my references were books, meta-analysis, or systematic reviews only two of the following articles were frequently used.

Reference: Berg V, Nøst TH, Sandanger TM, Rylander C. Predicting human plasma concentrations of persistent organic pollutants from dietary intake and socio-demographic information in the Norwegian Women and Cancer study. Environment International. 2018;121(Pt 2):1311-8.			**Study design: Cohort study** Grade - quality ⊕⊕⊕
Aim	Material and method	**Results**	**Discussion/comments**
To develop and evaluate statistical models for predicting plasma concentrations of persistant organic pollutants (POPs) in participants of the Norwegian Woman and Cancer Study (NOWAC), using information from questionnaires and measured plasma concentrations of POPs. **Conclusion** Predicted plasma concentrations of specific PCBs showed good prediction (Kw > 0.4) compared to measured concentrations. The models can therefore be used to classify the participants from NOWAC in groups based on high, medium and low exposure. **Land** Norway **Year of data collection** Questionnaires: 1991, 1998, 2004, 2005 Blood samples: Subsample 1 (model sample): 2005 Subsample 2 (validation sample): between 2003 og 2006	**Material:** Information on dietary intakes and sociodemographic variables from four different questionnaires from NOWAC **Population:** Participants of NOWAC **Cohorts:** Two subsamples - the model sample (n = 259) was used to create the prediction model, while the validation sample (n = 108) was used to validate the model. **Main outcome:** A prediction model that can be used to predict the plasma concentration of PCBs in NOWAC **Important confounding factors:** None reported **Methods:** POP concentrations were measured in 367 blood samples, and multivariable linear regression models were built for p.p´DDE, PCB-118, -138, -153, -180 and summed PCBs **Statistical methods:** Partial least squeare regressions Linear regression models Comparing of measured and predicted values: Correlation coeifficents. Evaluation of inter-method agreement: Weighted Cohen´s κ for tertile categorization.	**Main findings** Median POP concentrations in the population ranged from 13 ng/g lipid to 162 ng/g lipid (lowest for PCB-118 and highest for p,p′-DDE). Birth year, breastfeeding and the weight-related variables (BMI or weight change) were common predictors for all POPs. Influential dietary variables differed and were of varying importance. The predicted plasma concentrations were significantly correlated with the measured values (rs = 0.24, 0.33, 0.41, 0.50, 0.56, and 0.54 for p,p′-DDE, PCB-118, -138, 153, -180 and summed PCBs, respectively). Tertiles of predicted plasma concentrations displayed significant, but varying agreement with measured concentrations (Weighted Cohen's κ = 0.19, 0.22, 0.33, 0.42, 0.45, and 0.50 respectively). **Additional findings** A certain dietary pattern in the study: Women with a high intake of boiled cod were more likely to consume fish liver which has a high fat content (> 50%).	**Checklist** • Is the aim clearly formulated? **Yes** • Are the groups recruted from the same population (selection bias)? **Yes** • Were the groups comparable regarding important bacground factors (selection bias)? **Yes** • Were the exposed individuals representative of a defined population? **Yes** • Was exposure and outcome measured equally and validated in the two groups? (Classification bias) **Partially. The blood samples from the two subsamples were extracted and analysed at different locations: Model sample at NILU and Validation sample in Quebec Canada, leading to a higher reported concentrations of PCB-138 at NILU, due to coelution.** • Was the person validating the results blind to group affiliation? **No** • Was it a prospective study? **No** • Were important confounding factors accounted for in the design and analyses? **No** • Do you believe in the reults? Bradford Hills criteria (time sequence, dose- response gradient, biological plausibility, consistency….) **Yes** • Can the results be transferred to the general population? **Partially. It can be transferred to the NOWAC study which is a large cohort study considered as representative of the general population in Norway** • Other litterature that strengthens or weakens the results? • What does the results mean for change of practice? **Less expensive analysises and the ability to study larger populations when investigating PCBs** • What does the writers discuss as Strenghts: **Repeated information on sociodemographic and lifestyle variables, allowing them to study changes over time. PLS regressions simplified the overview of correaltion and minimized the use of multiple statistical comparison and therefor the probablitiy of wrongly concluding on statistically significant predictors.** Weaknesses: **Recall bias because of questionnaires, affecting precision and subsequent misclassification of participants. POP analyses performed at different laboratiories. Temporal trends in dietary habits and sociodemographic variables makes it difficult to transfer to other populations with different age or dietary patterns.**

Reference: Hjartåker A, Andersen LF, Lund E. Comparison of diet measures from a food-frequency questionnaire with measures from repeated 24-hour dietary recalls. The Norwegian Women and Cancer Study. Public Health Nutr. 2007;10(10):1094-103.			**Study design: Cohort study** (I had difficulties deciding the design, as it in some ways function as a validation study, as well as a diagnostic study if you consider the test/reference aspect. I chose to define it as a cohort study because it includes follow-up over time, is based on the cohort study NOWAC and included in the EPIC-studies Grade - quality ⊕⊕⊕
Aim	Material and method	**Results**	**Discussion/comments**
To compare diet measures from a food-frequency questionnaire (FFQ) with measures from 24-hour dietary recalls (24HDRs). **Conclusion** The FFQ's ability to rank subjects was good for foods eaten frequently and fairly good for macronutrients in terms of energy percentages. Weaker ranking abilities were seen for foods eaten infrequently and for some micronutrients. The results underline the necessity of performing measurement error corrections. **Land** Norway **Year of data collection** NOWAC: - First questionnaire: 1991-1992 - Second questionnaire: 1998 - 24HDRs: 2002 - Third questionnaire: 2003	**Population:** Of 500 women randomly selected from The Norwegian Women and Cancer Study (the Norwegian arm of the European Prospective Investigation into Cancer and Nutrition), 286 agreed to participate and 238 completed the study. **Main outcome:** Reproduction of food intake **Important confounding factors:** As a reward for completing all four interviews the participants were offered to participate in a lottery of 20 subscriptions for a weekly magazine for half a year The participants answered FFQs after completing the 24HRDS **Methods:** The participants answered an FFQ (test method) after completing four, repeated 24HDRs (reference method) during a year.. 24HDRs were performed via phone, and EPIC-SOFT. Four interviewers at UiO and six at UiT performed interviews. Half of them had nutritional background. Alternation system to reduce risk of systematic bias. Seasonal variation. **Statistical methods:** Wilcoxon signed rank test Difference-against-mean plots Spearman´s correlation coefficient Calibration coifficient calculated by regression of the 24HDR data on the FFQ data Linear regression model Residual plots	**Main findings** Othe FFQ overestimated absolute intake in seven and underestimated intake in six of 21 food groups. Intakes of energy, fat, added sugar and alcohol were lower in the FFQ than in the 24HDRs, Intake of fibre was higher. Spearman's rank correlation coefficient ranged from 0.13 (desserts) to 0.82 (coffee) for foods, and from 0.25 (β-carotene) to 0.67 (alcohol) for nutrients. 3 % of the observations on nutrient intake fell in the opposite quintile when classified according to the FFQ as compared with the 24HDR. The median calibration coefficient, calculated by regression of the 24HDR data on the FFQ data, was 0.57 for foods and 0.38 for nutrients. **Additional findings** 45 women did not complete the FFQ, suggesting that this type of study and nutrient recall is to tiring for the participants.	**Checklist** • Is the aim clearly formulated? **Yes** • Are the groups recruted from the same population (selection bias)? **Yes** • Were the groups comparable regarding important bacground factors (selection bias)? **Yes** • Were the exposed individuals representative of a defined population? **Yes** • Was exposure and outcome measured equally and validated in the two groups? (Classification bias) **Partially.** • Was the person validating the results blind to group affiliation? **No** • Was it a prospective study? **No** • Were important confounding factors accounted for in the design and analyses? **Ye** • Do you believe in the reults? Bradford Hills criteria (time sequence, dose- response gradient, biological plausibility, consistency….) **Yes** • Can the results be transferred to the general population? **Partially. It can be transferred to the NOWAC study which is a large cohort study considered as representative of the general population in Norway** • Other litterature that strengthens or weakens the results? • What does the results mean for change of practice? **Less time consuming analysises and the ability to study larger populations when investigating food intake** • What does the writers discuss as strengths and weaknesses. **The writers doesn´t adress the subject strengths or weaknesses, however did a thorough discussion where the following challenges are adressed:** ¼ of the interviews were regarded as covering a «special day». The FFQs were answered afte the 24HRDs. The number of recalls may not have been large enough. Data technical matters may have contributed to discrepancy. The definitions of different food stuffs were not specific enough. The reference method may underestimate dietary intake. Strengths: relative validity is comparable to FFQs used in other large cohort studies.

Reference: Aronson KJ, Miller AB, Hanna WM, Sengupta SK, Weber JP, Woolcott CG, et al. Breast Adipose Tissue Concentrations of Polychlorinated Biphenyls and Other Organochlorines and Breast Cancer Risk. Cancer Epidemiol Biomarkers Prev. 2000;9(1):55-63.			**Study design: Case-control** **Grade – quality** ⊕⊕
Aim	Material and method	**Results**	**Discussion/comments/checklist**
To evaluate the association between breast cancer risk and breast adipose tissue concentrations of several organochlorines . **Conclusion** Clear associations with breast cancer risk were demonstrated in this study for some PCBs measured in breast adipose tissue. **Land** Canada Year of data collection July 1995 to june 1997	**Population** Women under the age of 80 were enrolled by surgeons at Womens College Hospital in Toronto and Kingston General Hospital in Kingston where they were scheduled for excision biopsy of suspected breast cancer. Exclusion: previous diagnosis of any cancer except nonmelanoma skin cancer, breast implants, were participating in Tamoxifen trial or were too ill. 824 women, 735 agreed to participate. 663 completed questionnaire. **Case:** Subjects diagnosed with in situ or invasive breast cancer **Control:** Subjects with biopsies negative for malignancy, but most diagnosed with some form of BBD **Main exposure:** Organochlorines **Important confounding factors:** Age, study site, menopausal status ever pregnant, lactation, present use of HR, ethnicity, family history, BMI, fat intake, alcohol intake, present smoking, cumulative smoking **Method:** The biopsy tissue of 217 cases and 213 benign controls frequency matched by study site and age in 5-year groups was analyzed for 14 polychlorinated biphenyl (PCB) congeners, total PCBs, and 10 other organochlorines, including p,p*-1,1-dichloro2,2-bis(p-chlorophenyl)ethylene. Multiple logistic regression was used to assess the magnitude of risk **Statistical methods:** Multiple logistic regression Pearson correlation coeifficient Spearman correlation coeifficient Unconditional logistic regression	**Main findings** While adjusting for age, menopausal status, and other factors, odds ratios (ORs) were above 1.0 for almost all organochlorines except five pesticide residues. The ORs were above two in the highest concentration categories of PCB congeners 105 and 118, and the ORs for these PCBs increased linearly across categories (Ps for trend	Checklist: • Is the aim clearly formulated? **Yes** • Is Case-control design suitable for the objective? **Partially – the study looks at breast cancer risk, but doesn´t follow the participants over time and therefore doesn´t know if the controls develop breast cancer later in life** • Are the cases recruted in a «good» way? (Same period of time/grades of disease – selection bias*) **They were recruted before knowing if they were cases or controls** • Is the diagnose validated? (Classific. Bias) (prev/insi case) **It was validated after recrution** • Are the controls recruted in a «good» way? **Same way as cases** • Can disease in the control group be excluded (classific. Bias?) **No** • Were the case-control groups picked from comparable populations? **Yes** • Non-responders? Differences from case/control group? **72 women did not respond, difference is unknown because it was before diagnosis.** • Are the groups comparable regarding important background factors? **Cases were on average 4 years older than controls and therefore a higher proportion of cases were postmenopausal. Fewer cases than controls presently used HRT. More cases than controls had been pregnant, and although more cases had breastfed, they did it for a shorter time, and at older age. More cases were of British or Canadian ethnicity and had a family history of breast cancer in first or second degree relatives. Cases also had higher average dietary fat and BMI than controls, and more cases drank less than one alcoholic beverage per week and presently did not smoke** • Is main exposure validated? (Classific. Bias?) **Yes** • Are the groups treated the same? (detection bias?) **Yes** • Did the writers take into account important confounding factors in design/analysis? **Yes** • Is exposure to danger, damage, measures measured and graded the same in both groups? (classific. Bias?) **No** • Was the one who measured exposure/collected data blinded in regard to who were case/control? **Yes** • Do you believe in the results? **Yes** • Can the results be transferred to practice? **Yes, environmental prevention** • Are the findings supported by litterature? **Yes** • What is discussed as strenghts and weaknesses? **Strengths: breast adipose tissue, negative breast biopsy as control, more organochlorines than others. (minimizing two biases). Weakness: controls had BBD, noncomparable levels of organochlorines, Small population** • Does the results have plausible biological explanations? **Yes**

Reference: : Banks E, Beral, Bull D, Reeves G, Austoker J, English R, et al. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet. 2003;362(9382):419-27.			**Study design: Cohort study** Grade - quality ⊕⊕⊕
Aim	Material and method	**Results**	**Discussion/comments**
To investigate the relation between various patterns of use of HRT and breast cancer incidence and mortality.[11] **Conclusion** Current use of HRT is associated with an increased risk of incident and fatal breast cancer; the effect is substantially greater for oestrogen-progestagen combinations than for other types of HRT. **Land** UK **Year of data collection** 1996-2001	**Material/method:** Women invited for screening at 66 NHS breast screening units were sent a questionnaire to complete before they were screened; 71% of women screened participated: 1,084,110 women **Cohorts** Incident breast cancer Breast cancer deaths **Main outcome:** Prevalence of use of HRT **Important confounding factors:** Womens menopausal status, age, time since menopause, parity, age at firsth birth, family history of breast cancer, BMI, region of residence anad deprivation index. Allcohol comsumption, previous use of OC, age at menarche and past health. **Statistical methods:** Cox regression models Estimates of relative risk	**Main findings** Half the women had used HRT; 9364 incident invasive breast cancers and 637 breast cancer deaths were registered after an average of 2.6 and 4.1 years of follow-up, respectively. Current users of HRT at recruitment were more likely than never users to develop breast cancer (adjusted relative risk 1·66 [95% CI 1·58–1·75], p<0·0001) and die from it (1·22 [1·00–1·48], p=0·05). Past users of HRT were, however, not at an increased risk of incident or fatal disease (1·01 [0·94–1·09] and 1·05 [0·82–1·34], respectively). Incidence was significantly increased for current users of preparations containing oestrogen only (1·30 [1·21–1·40], p<0·0001), oestrogen-progestagen (2·00 [1·88–2·12], p<0·0001), and tibolone (1·45 [1·25–1·68], p<0·0001), but the magnitude of the associated risk was substantially greater for oestrogen-progestagen than for other types of HRT (p<0·0001). Results varied little between specific oestrogens and progestagens or their doses; or between continuous and sequential regimens. The relative risks were significantly increased separately for oral, transdermal and implanted oestrogen-only formulations (1·32 [1·21–1·45]; 1·24 [1·11–1·39]; and 1·65 [1·26–2·16], respectively; all p<0·0001). In current users of each type of HRT the risk of breast cancer increased with increasing total duration of use. 10 years' use of HRT is estimated to result in five (95% CI 3–7) additional breast cancers per 1000 users of oestrogen-only preparations and 19 (15–23) additional cancers per 1000 users of oestrogen-progestagen combinations. Use of HRT by women aged 50–64 years in the UK over the past decade has resulted in an estimated 20000 extra breast cancers, 15000 associated with oestrogen-progestagen; the extra deaths cannot yet be reliably estimated.	**Checklist** • Is the aim clearly formulated? **Yes** • Are the groups recruted from the same population (selection bias)? **Yes** • Were the groups comparable regarding important bacground factors (selection bias)? **Yes** • Were the exposed individuals representative of a defined population? **Yes** • Was exposure and outcome measured equally and validated in the two groups? (Classification bias) **Yes** • Was the person validating the results blind to group affiliation? **No** • Was it a prospective study? **Yes** • Were important confounding factors accounted for in the design and analyses? **Yes** • Do you believe in the reults? Bradford Hills criteria (time sequence, dose- response gradient, biological plausibility, consistency….) **Yes** • Can the results be transferred to the general population? **Partially. It can be transferred to the The Million Women Study study which is a large cohort study considered as representative of the general population in the UK** • Other litterature that strengthens or weakens the results? • What does the results mean for change of practice? **Taking precaucion when prescribing HRTs to women, especially if they have other risk factors of breast cancer** • **What does the** writers discuss as **The writers does not really discuss strenghts and weaknesses in this study, nor in the studies referred to that are supposed to describe the cohort Strenghts: misclassification should not affect main conclutions,** **Weaknesses: During follow-up some users may have becoma past users and never users may become current users**

Reference: Huang W, He Y, Xiao J, Huang Y, Li A, He M, et al. 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. 2019;26(31):32128-36.			**Study design: Case-control** (This article was only used as an example reference in the thesis) **Grade – quality** ⊕ Due to lack of information about cases and controls.
Aim	Material and method	**Results**	**Discussion/comments/checklist**
To evaluate the associations between adipose tissue PCB, DDT, and DDE concentrations and breast cancer risk. **Conclusion** This study suggests associations between the exposure of PCBs, p,p′-DDT, and p,p′-DDE and breast cancer risk. Based on adjusted models, PCB-118, PCB-138, PCB-153, PCB-180, ∑PCBs, and p,p′-DDE exposures increase breast cancer risk at current exposure levels, despite existing inconsistent even inverse results in PCB-28, PCB-52, PCB-101, and p,p′-DDT. More epidemiological studies are still needed to verify these findings in different populations. **Land** China Year of data collection 2014-2016	**Population** 209 pathologically diagnosed breast cancer cases and 165 controls were recruited from three local hospitals in Shantou city, China **Cases:** Undergoing surgery for newly diagnosed as invasive breast cancer who were histopathologically confirmed as cases **Control:** e histopathology confirmed as benign breast disease or non-breast-related disease would also provide breast or abdominal adipose tissue after undergoing surgery **Main exposure:** Organochlorines and PCB **Important confounding factors:** Older age, early menarche age, older age at first birth, family history of breast cancer, no lactation, and no parity were established to be risk factors for breast cancer, **Method:** Concentrations of 7 PCB congeners, p,p′-DDT, and p,p′-DDE were measured in adipose tissues obtained from the breast for cases and the breast/abdomen for controls during surgery. Clinicopathologic information and demographic characteristics were collected from medical records. PCBs, p,p′-DDT, and p,p′-DDE concentrations in adipose tissues were compared between cases and controls. Multivariate logistic regression model was used to analyze the risk of breast cancer by PCBs, p,p′- DDT, and p,p′-DDE concentrations in adipose tissues. **Statistical methods:** Multivariate logistic regression Cochrane-Mantel-Haenszel chi-square test.	**Main findings** Breast cancer cases have relatively higher menarche age, higher breastfeeding and postmenopausal proportion than controls. Levels of PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB-180, total PCBs (∑PCBs), and p,p′-DDE were relatively higher in breast cancer cases than controls. Breast cancer risk was increased in the third tertile of PCB-101, PCB-118, PCB-138, PCB-153, PCB-180, ∑PCBs, and p,p′-DDE as compared with the first tertile in both adjusted and unadjusted logistic regression models (odds ratios [ORs] were from 1.58 to 7.88); and increased linearly across categories of PCB-118 and p,p′-DDE in unadjusted model, and PCB-118 and PCB-153 in the adjusted model with trend (all $P < 0.01$). While breast cancer risk was declined in the second tertile of PCB-28, PCB-52, and PCB-101 in both unadjusted and adjusted models, also second tertile of p,p′-DDT and third tertile of PCB-28 in the adjusted models.	Checklist: • Is the aim clearly formulated? **Yes** • Is Case-control design suitable for the objective? **Partially – the study looks at breast cancer risk, but doesn´t follow the participants over time and therefore doesn´t know if the controls develop breast cancer later in life** • Are the cases recruted in a «good» way? (Same period of time/grades of disease – selection bias*) **Lacks information on how they were recruited** • Is the diagnose validated? (Classific. Bias) (prev/insi case) **Not sure** • Are the controls recruted in a «good» way? **Same as cases** • Can disease in the control group be excluded (classific. Bias?) **No** • Were the case-control groups picked from comparable populations? **Yes** • Non-responders? Differences from case/control group? **Not mentioned.** • Are the groups comparable regarding important background factors? The cases and controls have similar lifestyles and dietary habits, same ethnic and religious practices. **Cases have older menarche age than control** • Is main exposure validated? **Yes** • Are the groups treated the same? (detection bias?) **Not sure** • Did the writers take into account important confounding factors in design/analysis? **Yes** • Is exposure to danger, damage, measures measured and graded the same in both groups? (classific. Bias?) **No** • Was the one who measured exposure/collected data blinded in regard to who were case/control? **No** • Do you believe in the results? **Partially** • Can the results be transferred to practice? **No, further investigations are needed** • Are the findings supported by litterature? **Yes** • What is discussed as strenghts and weaknesses? **Strenghts: PCB and organochlorine pesticides measured in area with few studies on the subject. Only newly diagnosed breast cancer cases were included. Weaknesses: The measured PCB and organochlorine pesticide levels were after diagnosis and may not represent concentrations in special periods of early exposure. Controls were women with benign breast disease or non-breast related disease, and collected adipose tissues included normal tissues which may lead to imbalance. Breast cancer susceptibility genes are not analyzed.** • Does the results have plausible biological explanations? **Yes**