

Research article

Organochlorine exposures influence on breast cancer risk and survival according to estrogen receptor status: a Danish cohort-nested case-control study

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Abstract

Background: The relationship between breast cancer and organochlorine exposure is controversial and complex. As estrogen receptor positive and negative breast cancer may represent different entities of the disease, this study was undertaken to evaluate organochlorines influence on breast cancer risk and survival according to receptor status.

Methods: The background material stems from the Copenhagen City Heart Study (Denmark 1976-78). The breast cancer risk was investigated in a cohort nested case-control design including 161 cases and twice as many breast cancer free controls. The cases served as a cohort in the survival analysis. Serum organochlorine concentrations were determined by gaschromotography.

Results: The observed increased breast cancer risk associated with exposure to dieldrin derived from women who developed an estrogen receptor negative (ERN) tumor (Odds ratio [OR] I vs. IV quartile, 7.6, 95% confidence interval [95% CI] 1.4-46.1, p-value for linear trend 0.01). Tumors in women with the highest dieldrin serum level were larger and more often spread at the time of diagnosis than ERP tumors. The risk of dying was for the remaining evaluated compounds higher among patients with ERP breast cancer when compared to those with ERN. In the highest quartile of polychlorinated biphenyls (Σ PCB) it was more than 2-fold increased (Relative risk [RR] I vs. IV quartile, 2.5, 95% CI 1.1-5.7), but no dose-response relation was apparent.

Conclusion: The results do not suggest that exposure to potential estrogenic organochlorines leads to development of an ERP breast cancer. A possible adverse effect on prognosis of hormone-responsive breast cancers needs to be clarified.

Background

Many organochlorines have become ubiquitous in the environment and in the human body due to their resistance to degradation and their high solubility in lipids, which leads to accumulation in adipose tissue. Some of these compounds are suspected of disrupting the endocrine system and thereby increasing the risk of hormone-dependent disorders and diseases such as breast cancer [1–3]. Epidemiological studies indicate that total lifetime exposure to estrogen predict the risk of breast cancer [4,5]. Estrogen also plays an important role for the prognosis of breast cancer as treatment with the anti-estrogenic drug tamoxifen improves the survival of postmenopausal women with estrogen receptor positive (ERP) breast cancer [6]. Furthermore, estrogen stimulates proliferation of ERP breast cell lines and may therefore be associated with ERP human breast cancers only [7].

Previous studies of the potential effect of estrogenic organochlorines on breast cancer risk have yielded inconsistent results [8–23] and only a few has taken into account estrogen receptor status (ER) [16,17,19]. In a small Canadian study women with ERP tumors, but not those with estrogen receptor negative (ERN) tumors, had a higher DDE and PCB body burden compared to women with benign breast disease [16]. An excess risk of developing an ERP breast cancer associated with exposure to certain PCB congener was also demonstrated in a study conducted in Sweden [19].

In the so far only study on breast cancer survival, an adverse effect of exposure to dieldrin was identified, but ER was not obtained at that stage [24].

It has been postulated that ERP and ERN are two different entities of breast cancer having different biological and etiological mechanisms. Thus, distinguishing between breast cancer which growth is dependent on estrogen and those which is not i.e. ERP and ERN tumors, may have implications for studying potential estrogenic organochlorines possible influence on breast cancer risk and survival. Presented in this paper are results of analysis on breast cancer risk and overall survival while accounting for ER of the primary tumors.

To avoid problems with multiple comparisons, the analyses were restricted to organochlorines with estrogenic properties or compounds that are indicators of previous exposure to estrogenic ones.

Material and methods

The background material constitutes the Copenhagen City Heart Study (CCHS), which in 1976–78 enrolled a random sample of 10,317 women living within 10 wards

of the National University Hospital of Copenhagen, Denmark. This study was designed as a cohort-nested case-control study, where the case group consisted of 268 women between 25 and 80 years of age diagnosed with primary breast cancer between initiation of the CCHS and end of follow up in 1993. They were identified through computerized linkage to the Danish Cancer Registry using the unique 10-digit ID-number issued to each resident in Denmark. As controls, a random sample of 536 women matched on age, and vital statistics was selected from the remainder of the CCHS cohort. Information on lifestyle factors, reproductive history, and socioeconomic conditions was obtained through standardized questionnaires in connection with a physical examination, which included body weight, and non-fasting blood samples. The serum was analyzed for cholesterol, triglycerides, and the remaining volume frozen. After the 17 years of follow up stored serum was retrieved from 240 (89.5%) cases and 477 (89.0%) of the matched controls.

Information on vital statistics, date of death until July 31 1997, breast cancer characteristics, and adjuvant treatment, were achieved through linkage to the Civil Registration System, the Causes of Death Registry, and the nation-wide clinical breast cancer trial conducted by the Danish Breast Cancer Co-operative Group. The latter was also able to provide information on where each woman diagnosed with breast cancer was treated. This made it possible to contact the pathology departments where the tumor tissue had been reviewed. Paraffin embedded tumor tissue was requested for estrogen receptor status determination immunohistochemically by microwave antigen retrieval, a mouse monoclonal anti-estrogen receptor antibody ER1D5, and the streptavidin-biotin detection system [25]. The threshold for designation of estrogen receptor positivity was staining of $\geq 10\%$ of the cell nuclei in the specimen.

Breast cancer characteristics included tumor size, degree of spread, and stage of disease. Patients who were defined as having received adjuvant therapy with tamoxifen include those treated solely with tamoxifen, and those who had a combination therapy including tamoxifen.

The stored serum was analyzed for potential estrogenic organochlorine compounds and their metabolites by the U.S. Center of Disease Control and Prevention in Atlanta: Hexachlorobenzene (HCB), dieldrin, polychlorinated biphenyls (Σ PCB calculated as the sum of following congeners: IUPAC numbers 28, 52, 56, 66, 74, 99, 101, 105, 110, 118, 138, 146, 153, 156, 170, 172, 177, 178, 180, 183, 187, 189, 193, 194, 195, 201, 203, and 206), and p,p'-DDE. The analytical technique involves a two-stage sol-

id-phase extraction and clean-up followed by dual-column gas-chromatographic separation and electron capture detection [26]. To test for quality, bovine serum was used to which three concentrations of 9 commercial analytes were added. With every batch of 10 samples one quality control and one reagent-blank sample was analyzed. All quality control results were compared with limits established in the laboratory. If a quality sample was not within control limits the samples in this batch were discarded. Only completely resolved chromatographic peaks area ratios were compared with a linear calibration curve generated by analysis of standards of 6 different concentrations in triplet. Recoveries were calculated by subtracting the background level in the blank serum from the level in the spiked serum and dividing by the original spike concentration. The detection limit for all analytes was based on two SD of the result from the lowest control samples over the course of the analysis. The detection limits on the evaluated compounds are: HCB 0.05 ng/mL, dieldrin 0.15 ng/mL, p,p'-DDE 0.41 ng/mL, and varied for the PCB congeners from 0.13 ng/mL to 0.30ng/mL. For lipid adjustment the total serum lipid concentration (mg/dl) was calculated as:

$$2.27 \times \text{cholesterol (mg/dl)} + \text{triglycerides (mg/dl)} + 0.623 [27].$$

A total of 161 cases (67.1%) and their matched controls (318 breast cancer free women) were eligible for analysis of breast cancer risk according to ER. The case group (161) served as a cohort in the survival analysis.

Organochlorine concentrations were categorized in four levels of exposure using quartiles as cut-points. Associations between organochlorine exposure and breast cancer risk were examined by conditional logistic regression. ERP and ERN tumors were analyzed separately. The Cox proportional hazard method was used to investigate tumor characteristics, and the influence of organochlorine exposure on over all breast cancer survival [28]. The proportional hazard assumption was checked by log (-log) plots from stratified analyses. All variables included in the analyses complied with the assumption. Odds ratios (OR), relative risks (RR), and 95% confidence intervals (CI) were computed using SAS statistical software [29]. Body weight (kg, in quartiles), parity (0, 1, ≥ 2 children), menopausal status (pre- and postmenopausal), hormone replacement therapy (never, ever), smoking (never, ever), alcohol consumption (never or hardly ever, sometimes each month, sometimes each week, every day), household income (<4000DKK, 4000-10000DKK, >10000DKK per month before tax), and school education (<7, 7-10, >10 years) were included in the risk analysis. Tumor size (actual size in mm, or <50 mm, > 50 mm) degree of spread (axillary lymph

nodes with metastatic involvement: 0, > 1 nodes), and stage of disease (early stage: tumor size <50 mm and degree of spread 0 nodes; advanced stage: all other cases) were included in the survival analyses as potential confounders. Backward step-wise procedures were used to evaluate the modifying effect of the potential confounders and other organochlorines. Only covariates that reached statistical significance, were allowed to remain in the model. Due to the observations with non-detectable organochlorine concentrations, a test for trend in risk and prognosis was done using quartiles of organochlorine concentrations. All performed tests of statistical significance were two sided with a level of significance on 5%.

The study has been performed in accordance with the Helsinki Declaration and approved by the Scientific Ethical Committee serving Copenhagen and Frederiksberg Municipality (KF) 01-157/94. Computerized linkage between Danish registries was performed according to Danish legislation.

Results

The immunohistochemical estrogen receptor analysis showed that 72.0% (116 cases) had developed an ERP breast cancer and 28.0% (45 cases) an ERN cancers. Table 1 shows the measured lipid adjusted serum organochlorine concentration among these breast cancer cases.

Table 1: Lipid adjusted serum concentrations of organochlorines among breast cancer cases with available estrogen receptor status.

Organochlorine	Min – Max concentration (ppb) ^a	Median concentration (ppb)
HCB	LOD ^b – 1,901.50	269.75
Dieldrin	LOD – 345.69	28.30
ΣPCB	156.84 – 2,621.21	1,076.04
p,p'-DDE	162.01 – 5,072.56	1,129.75

^a Parts per billion; ^b Detection limit

High body weight, nulliparity, and use of hormone replacement therapy were identified as overall breast cancer risk factors (data not shown) and were therefore included as covariates in the analyses of organochlorine compounds. The excess risk of breast cancer associated with high body weight and hormone replacement therapy after menopause derives from women who developed and ERN breast cancer, but was not statistical significant (table 2).

Table 2: Breast cancer risk factors by estrogen receptor status.

Factor	Estrogen receptor positive		Estrogen receptor negative	
	Adjusted OR (95% CI) ^a	p trend ^b	Adjusted OR (95% CI)	p trend ^b
Weight (kg) by quartile				
<58	1.0 ref. ^c		1.0	
58-64	0.7 (0.5-1.5)		1.3 (0.5-3.6)	
65-71	1.1 (0.7-1.8)		1.9 (0.7-4.9)	
>71	1.2 (0.7-1.9)	>0.20	2.1 (0.8-5.9)	0.10
Number of full term pregnancies				
0	1.0		1.0	
1	1.0 (0.6-1.6)		1.7 (0.6-5.1)	
>1	0.6 (0.4-0.9)	0.01	1.2 (0.5-3.1)	>0.20
Hormone replacement-therapy				
Never	1.0		1.0	
Ever	1.1 (0.7-1.7)		1.8 (0.8-4.1)	

^a Odds ratio, 95% confidence interval, age-adjusted; ^b two sided p-value for linear trend in OR's, ^c reference category.

The median duration of follow up with regard to death of all causes was 7.2 years; a total of 125 breast cancer patients died.

The overall breast cancer survival was related to tumor size, degree of spread, and stage of disease (data not shown). These prognostic indicators were significantly associated with breast cancer tumors that were ERN (table 3).

Table 3: Over all breast cancer survival in relation to clinical characteristics and stage of disease by estrogen receptor status.

Characteristic	Estrogen receptor positive	Estrogen receptor negative
	Adjusted RR (95% CI) ^a	Adjusted RR (95% CI)
Tumor size (mm)		
<50	1.0 ref. ^b	1.0
≥ 50	0.7 (0.2-3.0)	5.4 (1.8-15.9)
Metastatic lymph nodes		
0	1.0	1.0
≥ 1	1.6 (0.9-2.7)	4.6 (1.7-12.3)
Stage of disease		
Early	1.0	1.0
Advanced	1.5 (0.9-2.6)	6.0 (2.1-16.9)

^a Relative risk of dying, 95% confidence interval, adjusted for age at diagnosis; ^b reference category.

Women with the highest serum concentration of dieldrin had a more than seven-fold increased risk of developing an ERN breast cancer compared to women with the lowest concentration, and the risk increased in a stepwise fashion with increasing dieldrin exposure level. No association could be observed for the ERP tumors (table 4). The frequency of large tumors (size >50mm) at the highest dieldrin quartile exposure group was 27.3% (average size, 37 mm) and 8.7% (average size, 27 mm) for ERN and ERP tumors, respectively. The corresponding figures for spread of disease was 61.5% and 45.8%.

For the remainder of the organochlorine compounds, ORs for the highest exposure level tended to be higher for women who developed an ERP breast cancer than those who had an ERN tumor, though none of the relationships reached statistical significance (table 4). In general, the risk of dying among women with the highest organochlorine exposure level was higher among women with ERP than ERN breast cancers, but the only statistical significant relationship was observed for ΣPCB (table 5). A statistically significant inverse trend was seen for exposure to HCB and ΣPCB in women with ERN tumors. Exposure to dieldrin was associated with a significant increased risk of dying for women with ERP tumors in the second and third quartile compared to the first quartile. An approximately 2-fold not significant increased risk of dying among women in the highest dieldrin exposure level compared to the lowest was observed for both ERP and ERN tumors (table 5). Similar results were obtained in analyses on a subgroup of 80 cases, where it was possible to adjust for tamoxifen therapy (data not shown).

Table 4: Risk of breast cancer in relation to serum concentrations of organochlorines in quartiles by estrogen receptor status.

Organochlorines in quartiles (ng/mL)	Estrogen receptor positive			Estrogen receptor negative		
	Cases/controls	Adjusted RR (95% CI) ^a	p trend ^b	Cases/controls	Adjusted OR (95% CI)	p trend
HCB						
I <206.50	36/56	1.0 ref. ^c		19/23	1.0	
II 206.50-269.75	20/55	1.1 (0.6-1.8)		8/23	0.5 (0.2-1.4)	
III 269.75-335.75	22/56	1.0 (0.6-1.7)		11/23	0.8 (0.3-2.2)	
IV >335.75	36/55	1.2 (0.7-2.1)	>0.20	6/23	0.4 (0.1-1.4)	>0.20
Dieldrin						
I <12.01	27/57	1.0		6/23	1.0	
II 12.01-28.30	28/56	1.3 (0.7-2.2)		5/23	1.2 (0.3-5.4)	
III 28.30-57.11	33/57	1.5 (0.8-2.7)		13/23	4.9 (0.9-28.3)	
IV >57.11	28/56	1.4 (0.8-2.5)	>0.20	20/23	7.6 (1.3-46.1)	0.01
ΣPCB						
I <811.11	36/57	1.0		14/23	1.0	
II 811.11-1076.04	24/56	1.1 (0.6-1.7)		11/23	1.0 (0.4-2.7)	
III 1076.04-1405.73	20/57	0.7 (0.4-1.2)		11/23	1.3 (0.4-3.9)	
IV >1405.73	36/56	1.3 (0.8-2.2)	>0.20	8/23	0.8 (0.3-2.6)	>0.20
p,p'-DDE						
I <741.04	33/57	1.0		14/23	1.0	
II 741.04-1129.75	29/56	0.7 (0.4-1.2)		11/23	0.9 (0.3-2.5)	
III 1129.75-1688.85	25/57	0.8 (0.5-1.3)		10/23	0.7 (0.2-2.0)	
IV >1688.85	29/56	0.9 (0.6-1.5)	>0.20	9/23	0.6 (0.2-1.7)	>0.20

^a Odds ratio, 95% confidence interval, adjusted for age, weight, parity, and hormone replacement therapy; ^b two sided p-value for linear trend in OR's; ^c reference category. Cases with missing information on confounders were excluded.

Including other organochlorines than the particular one in question in the multivariate analysis did not have any modifying effect on the observed estimates on breast cancer risk and survival.

The cases on whom a tumor tissue specimen could not be obtained were older at the time of entrance into the CCHS (>60 years of age, 42.3% vs. 19.1%), older at the time of diagnosis (>71 years of age at diagnosis, 32.1% vs. 19.1%), and more often nulliparous (nulliparity, 33.3% vs. 24.1%) compared to those with an available specimen.

Discussion

It has been postulated that ERP and ERN breast cancers represent different entities of the disease [7]. If this hypothesis is correct the risk factor profiles may differ between the two types of breast cancer, especially for hormone related factors as parity, hormone replacement therapy and body weight. The present study's results on these breast cancer risk factors according to ER are in accordance with previous epidemiological studies, which do not provide consistent evidence to conclude that development of ERP breast cancer is associated with exposure to estrogen related factors [30-36].

Only few prior studies have evaluated the role of potential estrogenic organochlorines relationship to breast cancer risk while accounting for estrogen receptor status of the primary tumors. An excess risk of developing ERP breast cancers associated with exposure to certain PCB congeners, and DDE have been demonstrated [16,19]. The present study could not confirm this. In fact, the previously reported excess risk observed with exposure to dieldrin derived from women who developed an ERN tumor [13]. However, it is still uncertain what happens in the preclinical phases of breast cancer development [37]. Low levels of available estrogen may stimulate estrogen receptor expression [38]. It is also possible that down-regulation of the estrogen receptor gene to immunohisto-chemically undetectable levels may occur in some tumors due to high circulating levels of estrogens [38]. A breast cancer initiated as an ERP cancer could therefore change receptor status during the process of carcinogenesis prior to diagnosis. Thus ER status may merely represent different stages in disease progress.

Table 5: Overall breast cancer survival in relation to serum concentrations of organochlorines by estrogen receptor status.

Organochlorines in quartiles (ng/mL)	Estrogen receptor positive			Estrogen receptor negative		
	Cases/control	Adjusted RR (95% CI) ^a	p trend ^b	Cases/controls	Adjusted RR (95% CI)	p trend
HCB						
I <206.50	13/15	1.0 ref. ^c		7/4	1.0	
II 206.50-269.75	15/14	0.7 (0.3-1.6)		8/3	0.9 (0.3-3.1)	
III 269.75-335.75	17/12	1.1 (0.5-2.4)		3/8	0.2 (0.0-1.2)	
IV >335.75	13/15	1.1 (0.5-2.5)	>0.20	4/7	0.2 (0.1-1.7)	0.03
Dieldrin						
I <12.01	8/21	1.0		4/7	1.0	
II 12.01-28.30	17/12	3.4 (1.3-8.7)		3/8	0.4 (0.1-3.1)	
III 28.30-57.11	17/12	2.6 (1.0-6.3)		7/4	1.9 (0.5-7.8)	
IV >57.11	17/12	2.2 (0.9-5.4)	>0.20	8/3	1.8 (0.3-5.5)	0.06
ΣPCB						
I <811.11	14/15	1.0		6/5	1.0	
II 811.11-1076.04	15/14	1.5 (0.6-3.4)		7/4	0.4 (0.1-1.6)	
III 1076.04-1405.73	13/16	1.0 (0.4-2.3)		5/6	0.5 (0.1-2.2)	
IV >1405.73	17/12	2.5 (1.1-5.7)	>0.20	4/7	0.1 (0.0-5.6)	0.02
p,p'-DDE						
I <741.04	15/14	1.0		6/5	1.0	
II 741.04-1129.75	13/16	0.7 (0.3-1.6)		4/7	0.3 (0.0-2.0)	
III 1129.75-1688.85	17/12	1.0 (0.5-2.2)		5/6	0.7 (0.1-3.7)	
IV >1688.85	14/15	1.0 (0.5-2.1)	>0.20	7/4	0.3 (0.1-2.1)	>0.20

^a Relative risk of dying, 95% confidence interval, adjusted for age at diagnosis, metastatic lymph nodes, and tumor size; ^b two sided p-value for linear trend in RR's; ^c Reference category. Cases with missing information on confounders were excluded.

Large tumors are more frequently heterogeneous with respect to estrogen receptor expression than small tumors [39–42]. Consequently, a greater proportion of the large tumors will not exhibit estrogen reactivity at the time of diagnosis, although some part of it will be ERP. The tissue specimen obtained at the time of diagnosis may therefore not necessarily reflect the original initiated breast cancer. These considerations may explain the present study's finding on dieldrin, as 27.3% of the ERN tumors among women in the highest exposure level had a diameter above 50 mm, while the corresponding figure for the ERP tumors was only 8.7%. Finally, it is possible that dieldrin is associated with a yet unidentified factor that modulates breast cancer risk.

Whether exposure to estrogen related risk factors or organochlorines with estrogenic potential results in development of an ERP breast cancer cannot be determined from current evidence, but deserves more attention in future larger studies.

In the only study evaluating the influence of organochlorines on overall survival of breast cancer, an adverse effect of dieldrin exposure was reported [24]. When the ER of the tumor was taken into account in the analysis of

dieldrin, the relative risk of dying in the highest exposure level was elevated in both patients with ERP and ERN tumors, though not significantly. An adverse effect of dieldrin on survival of women with ERP tumors could be anticipated, as this compound is able to stimulate the growth of human estrogen-sensitive cells [2]. Another non-hormonal mechanism must lie behind the poorer prognosis of women with ERN tumors. Possibly, exposure to dieldrin may lead to development of a tumor with an increased inherent aggressiveness beyond what this study could take into account i.e. tumor size, degree of spread, and stage of disease. The significantly poorer prognosis observed for ΣPCB in women with ERP tumors is in accordance with the hormonal potential of some PCB congeners [1].

The breast cancer's responsiveness to estrogen is exploited in endocrine surgery by removing the ovaries, and medically using drugs inhibiting the effects of estrogen on tumor cell growth. Worldwide results indicate that a 20% reduction in the five-year mortality is achieved by adjuvant therapy with tamoxifen in women over 50 years of age [6]. The use of tamoxifen has become more frequent and is not only reserved for postmenopausal patients or patients with ERP tumors, because a proportion

of up to 10% of ERN tumors is found to respond to the therapy as well [43,44]. In this study tamoxifen was preferably administered to postmenopausal patients, but 26.3% of the patients who received this treatment had an ERN tumor. Tamoxifen competes with endogenous estrogen in binding to the receptor, and may therefore also block a possible effect of an estrogenic compound. However, adjustment for tamoxifen therapy in survival analyses on a sub-sample of the cohort (80 cases) did not substantially alter the observed results. The present study has several strengths. The participants were selected independently of risk of breast cancer and were followed for 17 years with regard to development of the disease. This time span allows the breast cancer to develop and takes into account the presumed long latency of this cancer. Furthermore, it is possible to give a long-term estimate for overall survival, as the median follow up with regard to death was 7.2 years. The substantial amount of information collected on the participants in the CCHS and the linkage to several relevant registries assured the ability to adjust for potential confounding, when evaluating both breast cancer risk and prognosis. The observed differences between cases eligible for and not eligible for assessing ER are unlikely to have affected the findings on breast cancer risk. Parity would be expected to be associated with lower organochlorine concentrations, as lactation is a main route by which these substances are excreted. Younger age would mean shorter duration of exposure and less accumulation. Thus, the study subjects included in the present study could have lower serum organochlorine concentrations and slightly lower risk of breast cancer than the CCHS cohort in general. Exclusion of cases diagnosed within 5 years of serum sampling did not affect the present results on risk and survival, and neither did mutual adjustment for organochlorine compounds.

The Danish unique 10-digit ID-number issued to all persons living in and entering the country (by birth or immigration) insure the completeness of the undertaken linkages. Since 1942, the Danish Cancer Registry has registered all cases of cancer occurring in the entire Danish population; the registry is regarded virtually complete [45]. DBCG has since 1976 been notified about new breast cancer cases by all Danish hospital departments [46].

The present study was not designed as a classical survival study and blood samples for assessment of organochlorine exposure was taken on average 8.7 years before the patients were diagnosed with breast cancer. Given the changes in organochlorine concentrations over time, the study only indirectly addressed the question whether estrogen or estrogenic compounds determined in 1976-78 may interfere with the prognosis of ERP tumors. On the

other hand, breast cancer is estimated to take several (8-10) years to reach a clinical detectable size, which mean that assessment of organochlorine exposure at the time of diagnosis will not reflect the level at tumor initiation.

Even though this study included more breast cancer cases than most of the previous studies dealing with the organochlorine issue, it still has limited statistical power, especially in the analyses of ERN tumors.

Conclusion

The present study does not support the hypothesis that estrogen related risk factors or potential estrogenic organochlorines increase the risk of developing ERP tumors. In fact, it shows that exposure to dieldrin increases the risk of developing ERN tumors, which are larger and more often spread at the time of diagnosis. However, this finding should be interpreted with caution due to the limited number of ERN cases, so whether exposure to estrogenic organochlorine compounds affect the risk and prognosis of a hormone-responsive breast cancer needs to be clarified.

Competing interests: None declared

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