

DDT Exposure in Utero and Breast Cancer

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Context: Currently no direct evidence links in utero dichlorodiphenyltrichloroethane (DDT) exposure to human breast cancer. However, in utero exposure to another xenoestrogen, diethylstilbestrol, predicts an increased breast cancer risk. If this finding extends to DDT, it could have far-reaching consequences. Many women were heavily exposed in utero during widespread DDT use in the 1960s. They are now reaching the age of heightened breast cancer risk. DDT exposure persists and use continues in Africa and Asia without clear knowledge of the consequences for the next generation.

Hypothesis: In utero exposure to DDT is associated with an increased risk of breast cancer.

Design: This was a case-control study nested in a prospective 54-year follow-up of 9300 daughters in the Child Health and Development Studies pregnancy cohort ($n = 118$ breast cancer cases, diagnosed by age 52 y and 354 controls matched on birth year).

Setting and Participants: Kaiser Foundation Health Plan members who received obstetric care in Alameda County, California, from 1959 to 1967, and their adult daughters participated in the study.

Main Outcome Measure: Daughters' breast cancer diagnosed by age 52 years as of 2012 was measured.

Results: Maternal *o,p'*-DDT predicted daughters' breast cancer (odds ratio fourth quartile vs first = 3.7, 95% confidence interval 1.5–9.0). Mothers' lipids, weight, race, age, and breast cancer history did not explain the findings.

Conclusions: This prospective human study links measured DDT exposure in utero to risk of breast cancer. Experimental studies are essential to confirm results and discover causal mechanisms. Findings support classification of DDT as an endocrine disruptor, a predictor of breast cancer, and a marker of high risk.

Diethylstilbestrol (DES) is a synthetic estrogen, which was prescribed to pregnant women until it was banned in the United States in 1971 and is a seminal example of a transplacental carcinogen (1). The discovery that DES exposure in utero causes clear-cell carcinoma of the vagina and cervix (2) and also predicts higher risk for breast cancer (3) raises the possibility that other man-

made chemicals, particularly those that disrupt normal estrogen-related functions, could cause breast cancer in later life. Although DES (4) and other exogenous estrogenic chemicals (5, 6) have been shown to cause mammary cancer experimentally, no other in utero chemical exposures have been quantified and related prospectively to

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Abbreviations: CCR, California Cancer Registry; CERLab, Clinical and Epidemiologic Research Laboratory; CHDS, Child Health and Development Studies; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DDTs, DDT compounds such as the isomers *p,p'*-DDT and *o,p'*-DDT; DES, diethylstilbestrol; DMV, Department of Motor Vehicles; OR, odds ratio.

risk of breast cancer in a human population. The present study addresses this gap.

The influence of these in utero exposures in cancer risk informed an Endocrine Society scientific statement emphasizing that the timing of an exposure either to a hormone or an endocrine disruptor determines its effects (7). For this reason, human studies of adult exposure to estrogenic chemicals in relation to breast cancer risk are not sufficient (8, 9). Indeed, prior studies that investigated the association between midlife exposure to the pesticide dichlorodiphenyltrichloroethane (DDT) and breast cancer were largely negative (10).

We reported the one prior prospective and quantitative study to consider the implications of young age at DDT exposure on breast cancer risk. This study was based on incident breast cancer diagnosed before age 50 years in the mothers' generation of the Child Health and Development Studies (CHDS) pregnancy cohort over a 17-year follow-up (11). We used age in 1945, when DDT was widely introduced into the United States as a proxy for earliest age at DDT exposure to evaluate whether there was an increased susceptibility to breast cancer among women with early life exposure to DDT. We found that mothers who were exposed to DDT prior to age 14 years showed a 5-fold increase in risk of breast cancer (66th percentile of serum p,p'-DDT compared with the 33rd percentile); risk was greatest for women who were exposed even earlier, by age 4 years, and the DDT association with breast cancer was observed only for women who were exposed prior to age 14 years ($P = .02$ for interaction between p,p'-DDT and age in 1945). These findings clearly support the need to further investigate the role of early-life DDT exposure in later breast cancer risk. Other than this study, there is an absence of human breast cancer studies of in utero exposure to the DDTs (DDT compounds such as the isomers p,p'-DDT and o,p'-DDT that are found in technical DDT and also compounds that are breakdown products of DDT such as p,p'-dichlorodiphenyldichloroethylene (DDE), the most prevalent and persistent metabolite of p,p'-DDT) (12) due in part to the logistic barriers of quantitative assessment of gestational exposure coupled with follow-up of at least 50 years needed to identify breast cancer cases (13), given that the median age at diagnosis is 61 years (14).

DDT remains relevant to living populations for numerous reasons. First, most women born while DDT was extensively used worldwide are still alive today and are hence at risk for breast cancer. Second, DDT remains in active use for control of malaria in Africa and Asia in accordance with World Health Organization recommendations despite intense debate (15, 16). Third, because of its persistent presence in the environment, people worldwide con-

tinue to be exposed to DDTs that are already present (17). Environmental contamination and human exposure are greater where use was recent and where safer use, storage, and disposal are challenging (18), such as in China (19) and areas in Mexico (20, 21) and Africa (22, 23). Indeed, DDT health effects will remain relevant for the foreseeable future, given that the distribution of malaria vectors is predicted to expand with climate change (24). Melting glaciers release DDTs into arctic waters in which it is known to bioaccumulate to 1 million-fold higher concentrations in people, reaching levels found among populations with endemic malaria in tropical regions in which DDT use remains in effect (25, 26).

Here we conducted the first prospective study to relate quantitative measures of in utero DDT exposure to risk of breast cancer in daughters.

Materials and Methods

Subjects

This unique study is made possible by a 54-year follow-up of 20 754 pregnancies, resulting in 9300 live-born female offspring in the CHDS pregnancy cohort.

The CHDS was designed to examine the association between prenatal exposures and health and development over the life course for parents and children. The CHDS recruited women residing in the area of Oakland, California, who were members of the Kaiser Foundation Health Plan and received obstetric care for pregnancies between 1959 and 1967 (27). More than 98% of all eligible women enrolled. CHDS founding mothers voluntarily participated in an in-person interview and gave permission to researchers for medical record access for themselves and their children. Their blood specimens were collected at several times through pregnancy and 1–3 days after delivery. The present study was reviewed and approved by the Institutional Review Board of the Public Health Institute (Oakland, California), and we have complied with all federal guidelines governing use of human subjects.

Breast cancer cases

All members of the CHDS cohort are linked to the California Department of Motor Vehicles (DMV) files on a regular basis to determine California residence history, allowing us to update any name changes. All names registered with the DMV are used in establishing a match. Simultaneous linkage of multiple family members enhances matching. The regular DMV matching provides a history of location for each subject, which is used to determine the population at risk for cancer, corresponding with geographic surveillance by California's cancer registries.

Breast cancer cases were identified by linkage to the California Cancer Registry and the California Vital Status Records as previously described (11, 28) and by self-report during a survey of CHDS daughters conducted from 2010 to 2013. All names for each CHDS subject were submitted for cancer linkages using fixed (ie, birth date, sex, race, and name) and changeable (ie, address and patient record number) identifiers. A rigorous protocol was used to verify cases, comparing fixed vs changeable

identifiers by manual review. The California Cancer Registry (CCR) is reported to be greater than 99% complete after a lag time of about 2 years (29). We ascertained 80% ($n = 94$) of the cases via CCR linkage as of 2012 and 20% ($n = 24$) via self-report as of 2013. Due to the CCR lag time, the self-reported cases are more recently diagnosed than those from CCR. Tumor characteristics were available for 87% of CCR cases and for 50% of self-reported cases. Thus, we expect to have more complete information on tumor pathology with continuing CCR linkage.

Cases were defined as CHDS daughters with incident invasive or noninvasive breast cancer diagnosed by age 52 years, identified through surveillance and through self-report through March of 2013. There were 137 cases who met this case definition, diagnosed as of 2012. To be included in the present study, cases were required to have a maternal perinatal blood sample for measurement of DDT exposure, resulting in inclusion of 118 cases (86%). Three controls, matched on birth year and trimester of maternal blood draw, were selected at random for each case from among those who were under cancer surveillance and known to be free of breast cancer at the age of diagnosis for the matching case. Inclusion in the present study also required available data for the following variables known to be correlated with DDT exposure and potentially daughters' breast cancer: maternal lipids, age, race, early pregnancy weight, height, and history of breast cancer and whether the daughter was breast-fed, resulting in a final sample size of 103 cases (87% of those with serum samples) and 315 controls (83% of those with serum samples).

Serum assays

In 2014, we measured DDTs and serum lipids in nonfasting maternal perinatal serum samples that had been collected from 1959 through 1967. The mean age of subjects when blood was drawn was 26.9 years. We preferred to use the early postpartum samples (collected within 1–3 d after delivery) when available to conserve serum for future studies when timing within pregnancy is more critical. Early postpartum samples were available and used for most case-control strata (77.7%); third-trimester samples were used for 17.7%; second-trimester samples were used for 3.8%; and first-trimester samples were used for 0.7%. Prior work has established that organochlorine levels are consistent across all trimesters of pregnancy and soon after delivery within women (30). Serum samples had been stored at -20°C and were first thawed to prepare an aliquot of 1.5 mL for organochlorine assays. Aliquots were then shipped frozen to the laboratory of the California Department of Toxic Substances Control where they were assayed for DDTs, including *p,p'*-DDT and *o,p'*-DDT, the primary constituents of technical DDT, and the primary metabolite of *p,p'*-DDT, DDE using methods developed previously (31).

Briefly, human serum samples (1 mL) spiked with surrogate standards (tetrachloro-*m*-xylene, polychlorinated biphenyls-14, -65, and -166) were denatured with formic acid, extracted using Oasis HLB SPE cartridges (Waters Corp) and subsequently cleaned up with 33% sulfuric acid silica using an automated sample extraction system (RapidTrace; Biotage). DDT compounds (*o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE) were analyzed on a DB-5ms column (30 m \times 0.25 mm inner diameter, 0.25 μm film thickness; Agilent Technologies) installed in an Agilent gas chromatograph-tandem mass spectrometer (7890/7000B series). Chromatographic conditions included pulsed splitless in-

jection at 250°C and helium carrier gas at 1 mL/min. The gas chromatograph temperature program started with an initial temperature of 90°C , a hold for 1 minute, a ramp of $50^{\circ}\text{C}/\text{min}$ to 150°C , a hold for 1 minute, a ramp of $8^{\circ}\text{C}/\text{min}$ to 225°C , a hold for 6.5 minutes, and a final ramp of $14^{\circ}\text{C}/\text{min}$ to 310°C , a hold for 6 minutes. The mass spectrometer was operated in electron impact ionization mode using multiple reaction monitoring, source temperature of 275°C , ionization energy of 70 eV, and mass resolution of 1.2 amu. A calibration curve, consisting of five to eight standards with concentrations ranging from 0.1 to 30 $\text{pg}/\mu\text{L}$ (DDTs) or from 0.1 to 800 $\text{pg}/\mu\text{L}$ (DDE) and an R^2 value of 0.990 or greater, was used for quantitation.

Each batch of 10 samples was analyzed using a standard quality assessment and control protocol: a laboratory method blank (HyClone bovine serum; Fisher Scientific), a matrix spike in bovine serum, and a standard reference material (1958; National Institute of Standards and Technology). Matrix spike recoveries from bovine serum for *p,p'*-DDE, *p,p'*-DDT, and *o,p'*-DDT congeners ranged from $93\% \pm 14\%$, $102\% \pm 19\%$, and $101\% \pm 12\%$, respectively. Precisions from 51 standard reference material samples were reasonable, eg, coefficients of variation were 12%, 19%, and 14% for *p,p'*-DDE, *p,p'*-DDT, and *o,p'*-DDT, respectively. The method detection limits, calculated as 3 times the SD of the concentrations in method blanks ($n = 51$), were 0.013 ng/mL *o,p'*-DDT, 0.054 ng/mL *p,p'*-DDT and 0.158 ng/mL *p,p'*-DDE. Sample order was randomly assigned within and across batches. Case-control strata were analyzed in the same batches to minimize differences due to laboratory drift. The laboratory was blind as to case or control status of the samples. Interbatch and intrabatch coefficients of variation were 15% and 5% for *p,p'*-DDT, 6% and 5% for *p,p'*-DDE, and 11% and 7% for *o,p'*-DDT, respectively. The proficiency of the laboratory is demonstrated through regular successful participation in the Arctic Monitoring and Assessment Program (32) testing rounds ($z\text{-score} < 1$).

Using 150 μL undiluted serum, total cholesterol and triglycerides were measured enzymatically on a Roche P Modular system using reagents and calibrators from Roche Diagnostics at the Clinical and Epidemiologic Research Laboratory (CERLab) at Boston Children's Hospital, which is certified by the Centers for Disease Control and Prevention/National Heart, Lung, and Blood Institute Lipid Standardization Program. For cholesterol the method combines the specificity of the enzymatic reaction with peroxidase/phenol-4-aminophenazone indicator reaction (33). Cholesterol esters are hydrolyzed by cholesterol esterase to produce free cholesterol. In the presence of oxygen and cholesterol oxidase, cholesterol is oxidized to cholest-4-en-3-one and H_2O_2 . The latter product then reacts with a dye to generate a quinoneimine dye. The intensity of the generated color is measured at 505 nm and is directly proportional to the concentration of cholesterol in the measured sample. At cholesterol concentrations of 132.8 and 280.4 mg/dL, the day-to-day reproducibility in the CERlab, reflected by coefficient of variation, is 1.7% ($\text{SD} = 2.4$ mg/dL) and 1.6%, respectively ($n = 693$).

Triglycerides were measured enzymatically with correction for endogenous glycerol (34). In a preliminary reaction, the endogenous glycerol is phosphorylated in the presence of glycerol kinase and ATP. The formed glycerol-3-phosphate is oxidized to generate H_2O_2 , which reacts with 4-chlorophenol to produce an oxidative product. Then in the actual assay reaction, triglycerides are hydrolyzed by lipase mixture to generate glycerol and fatty acids. Similarly to the preliminary reaction, glycerol is phos-

phorylated by the action of glycerol kinase and the generated glycerol-3-phosphate is oxidized to produce H_2O_2 . The latter product reacts with a dye to generate a colored product. The intensity of the generated color is measured at 505 nm and is directly proportional to the concentration of triglycerides in the measured sample. Triglycerides at concentrations of 84.0 and 201.8 mg/dL are determined in the CERLab with a day-to-day reproducibility of 1.8% (SD 1.6 mg/dL) and 1.7% (SD 3.5 mg/dL), respectively (n = 675).

Statistical analysis

Data analyses were performed using age-matched, conditional logistic regression. Each DDT variable (o,p'-DDT, p,p'-DDT, and p,p'-DDE) was categorized in quartiles of the logged distribution based on the control population and represented as three nominal variables in models: quartile 2, quartile 3, and quartile 4 in which quartile 1 was the reference category.

We used a likelihood ratio criterion ($P < .05$) to choose the best model from among the following nested models: 1) all three DDT compounds were entered into the model, 2) terms for one of the three compounds was deleted beginning with the compound showing the highest P values and smallest effect sizes, and 3) models with only one DDT compound included. Trends across quartiles of DDT compounds in the best model were tested using natural log-transformed continuous variables. All models were adjusted for serum total cholesterol and total triglycerides entered as natural logs, maternal age (continuous), race (African American vs non-African American), overweight in early pregnancy (coded as overweight vs not, based on a body mass index ≥ 25 kg/m²), parity (primiparous vs multiparous), maternal history of breast cancer (yes vs no), and whether the daughter was breast-fed. The final model deleted adjustment variables that had little or no influence on the DDT predictors as evidenced by less than 10% change in the DDT coefficient(s) when removed from the model. Saturated interaction models were also tested for the three DDT variables (coded as continuous log transformed variables) and for the DDT variables in the final model with each

potential confounder. These were not significant and are not reported.

Results

Distributions of study variables are shown in Table 1. There were significant correlations among the DDT variables. However, the correlation of o,p'-DDT with p,p'-DDT was higher (Spearman rank correlation coefficient 0.78) than the correlation of either of these compounds with p,p'-DDE (0.60 and 0.66, respectively). Comparison of DDT associations in nested models are shown in Table 2, in which the best model included o,p'-DDT and p,p'-DDE (model 4, Table 2). Independent of a maternal history of breast cancer, elevated maternal serum o,p'-DDT significantly predicted a nearly 4-fold increase in the daughter's risk of breast cancer (Table 3). Maternal overweight in early pregnancy was associated with a lower risk of breast cancer in daughters but with marginal statistical significance. Maternal lipids, age, race, and parity and whether the daughter was breast-fed did not confound these findings and were not significant predictors of daughters' breast cancer. No interactions were statistically significant.

Most cases in this cohort were estrogen receptor positive (83%), progesterone receptor positive (76%), and HER2-negative (74%). In human breast cell lines, DDT activates HER2, a clinically relevant protein expressed in some breast cancers (35, 36). Therefore, we evaluated whether in utero DDT exposure was associated with HER2-positive breast cancers in this cohort of women

Table 1. Maternal Variables by Daughters' Breast Cancer Status

Maternal Variables	Controls (n = 315)			Cases (n = 103)		
	Percentile			Percentile		
	25th	50th	75th	25th	50th	75th
Age, y	22	26	30	23	27	31
o,p'-DDT, ng/mL	0.27	0.46	0.78	0.32	0.52	1.06
p,p'-DDT, ng/mL	8.38	12.98	18.57	9.47	13.18	20.11
p,p'-DDE, ng/mL	29.29	42.81	57.56	30.91	43.13	58.08
Cholesterol, mg/dL	182	222	278	188	227	283
Triglycerides, mg/dL	139	183	227	136	182	227
	Percent		n	Percent		N
History of breast cancer ^a	4.44		14	20.39		21
Primipara	29.84		94	26.21		27
African-American	25.08		79	23.30		24
Overweight (BMI ≥ 25 kg/m ²)	25.08		79	17.48		18
Breast-fed her daughter	26.03		82	31.07		32

Abbreviation: BMI, body mass index.

^a $P < .0001$ for difference between controls and cases.

Table 2. DDT Results for Nested Models

	o,p'-DDT	p,p'-DDT	p,p'-DDE
	OR	OR	OR
Univariate models			
Model 1, o,p'-DDT			
Quartile 2	1.8	–	–
Quartile 3	1.6	–	–
Quartile 4	2.8 ^a	–	–
<i>P</i> trend	0.053	–	–
Model 2, p,p'-DDT			
Quartile 2	–	1.9	–
Quartile 3	–	1.5	–
Quartile 4	–	2.2 ^b	–
<i>P</i> trend	–	0.074	–
Model 3, p,p'-DDE			
Quartile 2	–	–	1.3
Quartile 3	–	–	1.1
Quartile 4	–	–	1.3
<i>P</i> trend	–	–	NS
Model 4, o,p'-DDT plus p,p'-DDE			
Quartile 2	2.0	–	1.0
Quartile 3	1.8	–	0.7
Quartile 4	3.7 ^c	–	0.7
<i>P</i> trend	0.048	–	NS
Model 5, p,p'-DDT plus p,p'-DDE			
Quartile 2	–	2.0	1.1
Quartile 3	–	1.7	0.8
Quartile 4	–	2.9 ^d	0.8
<i>P</i> trend	–	0.037	NS
Model 6, o,p'-DDT plus p,p'-DDT plus p,p'-DDE			
Quartile 2	1.9	1.7	1.0
Quartile 3	1.7	1.2	0.7
Quartile 4	3.5 ^e	1.4	0.6
<i>P</i> trend	NS	NS	NS

Abbreviation: NS, not significant. All models are adjusted for maternal cholesterol and triglycerides, maternal overweight in early pregnancy, and maternal history of breast cancer. *P* trend is based on continuous log-transformed DDTs. Model 4 is the best model because deletion of p,p'-DDT (model 4 vs model 5) does not impact other associations, but deletion of p,p'-DDE (model 1 vs model 4) decreases the point estimate for o,p'-DDT. Although p,p'-DDE is not itself significant, it is inversely associated with daughters' breast cancer while being positively correlated with o,p'-DDT. NS, *P* > .15.

^a *P* = .007.

^b *P* = .058.

^c *P* = .004.

^d *P* = .054.

^e *P* = .019.

with available stage at diagnosis (73%) and available HER2 status (59%).

We found that o,p'-DDT was significantly, positively associated with advanced stage at diagnosis (regional or distant disease vs local or in situ) and with the occurrence of HER2-positive tumors, independent of maternal overweight, history of breast cancer, and p,p'-DDE. Despite small sample sizes for these analyses, 22 advanced-stage tumors and 16 HER2-positive tumors, respectively, results were statistically significant. The estimated odds ratio (OR) for diagnosis at an advanced stage was 2.2 [95%

Table 3. Maternal Predictors of Daughters' Breast Cancer

	OR	95% CI	<i>P</i> Value
History of breast cancer (yes vs no)	6.4	2.8–14.3	<.0001
Overweight in early pregnancy (yes vs no)	0.6	0.3–1.1	.077
o,p'-DDT (reference category is quartile 1)			
Quartile 2	2.0	0.9–4.3	.083
Quartile 3	1.8	0.8–4.0	.160
Quartile 4	3.7	1.5–9.0	.004
<i>P</i> trend	0.048		

Quartile cut points are given in Table 1. This table is based on a single model adjusted for variables shown and also adjusted for the maternal serum p,p'-DDE, total cholesterol, and total triglycerides, which were not significant predictors. Test for trend was based on log-transformed o,p'-DDT entered as a continuous variable instead of quartile variables.

confidence interval (CI) 1.1–4.2, *P* = .02] for a doubling of o,p'-DDT. The estimated OR for a HER2-positive tumor was 2.1 (95% CI 1.0–4.8, *P* = .05) for a doubling of o,p'-DDT. Levels of o,p'-DDT not only doubled but also tripled for women in the fourth quartile of the study compared with those in the first quartile (as seen in Table 1). The corresponding risk of advanced-stage and HER2-positive breast cancer for these women is more than 4-fold (for late stage, OR 4.6; 95% CI 1.3–16.5 for the fourth quartile of o,p'-DDT vs the first quartile; for HER2 positive, OR 4.6, 95% CI 1.1–19.7). These results suggest a strong effect of in utero o,p'-DDT on breast cancer stage, and HER2 status in this population, and the relevance of these findings is discussed below.

Discussion

Strengths

Exposure timing

Human and animal evidence establish the existence of developmental windows when the breast is more vulnerable to xenoestrogens, such as DDT (9). One of these windows is in utero. The present study is the first to quantify exposure to DDT in utero and link it to subsequent breast cancer risk.

Quantifying exposure

DDT was introduced in the general population in the United States in 1945 and was most heavily used worldwide in the late 1950's and 1960's (37). The CHDS enrolled pregnancies during 1959–1967, which, by coincidence, covered the years of highest DDT exposure (26,

38). Consistent with these data, the CHDS maternal serum samples, which were collected during the peak years of DDT use in the United States, show the highest levels of DDT compared with other studies of breast cancer in which samples were collected in later decades (11, 13). Thus, the present study uses unique historic samples to quantify typical in utero exposure when DDT use was at its peak. Our laboratory methods were able to quantify three DDT target compounds in all samples, including the lower concentration *o,p'*-DDT, a 15%–20% contaminant of technical DDT. This strength is due to state-of-the-art laboratory methods, which optimized the detection of all compounds, but is also due to collection of serum samples in the 1960's during a time period when there was high use of technical DDT.

Relevance to countries in which DDT is now banned

DDT was banned in the 1970's in the United States and western Europe (39). However, the women exposed most heavily while in utero during the 1960's are currently reaching the age of heightened breast cancer risk. Recent cases of breast cancer in daughters (F1) in the CHDS cohort represent the leading edge of birth cohorts heavily exposed to DDT when in utero. Thus, the findings of this present study are relevant to breast cancer, even in countries in which DDT is not currently used. In addition, DDT remains a global environmental contaminant due to its environmental persistence and semivolatility (25).

Relevance to countries in which DDT is still used

In those countries that continue to use DDT to control malaria, human exposure remains high (40). Thus, the findings of this study are relevant to current populations in which high in utero exposure is certainly occurring, such as in South Africa (23). The impact of DDT use on unborn generations has been recently raised as an ethical consideration (41). Our findings are relevant to this debate.

Plausibility

DDTs have been studied as possible endocrine disruptors on the basis of observed deleterious reproductive effects in wildlife (39, 42) and based on demonstrated endocrine-active effects including estrogen activity, particularly for *o,p'*-DDT (43, 44). Remarkably, independent of estrogen, there is likely a biological basis for the putative association of in utero DDT and HER2-positive cancers (36, 45, 46). For example, low-dose *o,p'*-DDT (1 nM) enhanced the tyrosine kinase activity of HER2 in human MCF-7 breast cancer cells irrespective of tamoxifen exposure or estrogen depletion of culture media (36, 46). *o,p'*-DDT also gave rise to increased MCF-7 foci (ab-

normal concentric piling up of cells in postconfluent cultures) (46). The effects of *o,p'*-DDT on tyrosine kinase activity of HER2 and MCF7 foci formation were blocked by a mononuclear antibody specific to HER2. These experimental results are consistent with the hypothesis this paper supports that *o,p'*-DDT can cause human mammary tumor formation that depends on the activation of HER2 (36, 46). Although this hypothesized effect of in utero DDT exposure on mammary cancer has not yet been tested experimentally in vivo, when exposure to its metabolite *p,p'*-DDE was initiated at weaning, the latency of HER2-positive mouse mammary tumors was shortened (47). Our nested pilot analysis also suggested that high exposure was significantly associated with advanced stage. Although no in vivo studies have evaluated this, a recent study of human breast cancer cell lines suggested *o,p'*-DDT caused estrogen-dependent invasion (48). Prioritizing the acquisition of tumor blocks and their immunohistochemical analysis of estrogen receptor and HER2 in the ongoing CHDS and in parallel experimental models will likely aid in resolving which receptor is the mechanistic target of in utero DDT on mammary carcinogenesis.

DES is the most well-studied perinatal xenoestrogen exposure in humans and is known to be associated with increased risk of breast cancer in both F0 (exposed in pregnancy) (49) and F1 (exposed in utero) (3). Similar to DES, we found that maternal serum *p,p'*-DDT was associated with an increased risk of breast cancer in CHDS mothers (F0) (11). We used birth year as a proxy for earliest age at DDT exposure and found the strongest association was observed in mothers who were initially exposed to DDT before the age of 4 years (11). The present study provides a direct, quantitative measure of exposure in utero for daughters (F1) in the CHDS and also finds an association between in utero DDT exposure and breast cancer.

Limitations

Outcome window

The present study investigates breast cancer diagnosed before age 52 years. Thus, these results do not address DDT associations with breast cancer diagnosed at a later age. One prior study of dioxin exposure to girls and women after a chemical explosion in Seveso, Italy, reported a significant association for breast cancers diagnosed within the first 20 years, which then declined over the subsequent 10 years of follow-up (50). Continuing follow-up in our cohort will be required to determine whether in utero DDT associations with breast cancer are observed for cases diagnosed in the future.

Exposure window

Whereas we have measured exposure directly relevant to fetal life, we cannot rule out a contribution of postnatal exposure. We did not observe a contribution or synergy with breast-feeding, even though this is a major maternal route of DDT exposure to offspring. However, this negative association may be partly explained by the low frequency of breast-feeding in the CHDS and the relatively small sample size.

Unmeasured confounders

We cannot rule out a contribution of other exposures that are correlated to DDT, including other unmeasured DDT metabolites. However, we were able to account for the DDTs that are present in the highest concentration in women.

Small sample

This study investigates the first cases observed in CHDS daughters. Although the sample size was adequate to observe a sizable association with in utero DDT, we are unlikely to have had the power to observe smaller associations with other risk factors or synergy among variables. To date, incomplete data on tumor pathology limits the interpretation of the correlation between DDT and tumors with more aggressive features.

Summary

We observed a sizable, statistically significant association between in utero DDT exposure and risk of breast cancer in young women and a possible association with more aggressive tumors. These findings are the first ever reported for a prospective observation of a large pregnancy cohort. Experimental studies are essential to confirm findings and discover causal mechanisms. If confirmed, these findings could lead to discovery of biomarkers and interventions for DDT-associated breast cancer. Our findings are relevant to the international debate on the costs and benefits of DDT use for malaria control.

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