Pesticides and Breast Cancer Risk: A Review of DDT, DDE, and Dieldrin

Suzanne M. Snedeker
Program on Breast Cancer and Environmental Risk Factors in New York State, Cornell University, Ithaca, New York, USA

Established risk factors for breast cancer explain breast cancer risk only partially. Hence, there has been interest in evaluating what role environmental chemicals, especially those with evidence of being hormonally active agents, play in breast cancer risk. Organochlorine pesticides have received the most attention because of their persistence in the environment, ability to concentrate up the food chain, continued detection in the food supply and breast milk, and ability to be stored in the adipose tissue of animals and humans. Although several early descriptive studies and a cohort study identified a strong positive association with breast cancer risk and adipose or blood levels of the organochlorine pesticide dichlorodiphenyltrichloroethane (DDT) and/or its metabolite dichlorodiphenyldichloroethylene (DDE), most of the more recent case-control and nested case-control studies have not supported this association. In this review I discuss these findings and explore how exposure to different forms of DDT with varying estrogenicities may have affected the results of these studies. I also address how other factors influence the interpretation of the studies on DDT, DDE, and breast cancer risk. These include the effect of analytic methods, dietary factors, menopausal status, use of different types of control populations, lactation history, estrogen receptor status, ethnic/racial subgroups, breast tumor characteristics, and polymorphisms. I also discuss the emerging research on whether serum levels of the persistent organochlorine insecticide dieldrin are related to breast cancer risk in Danish and American women. Further research needs are also identified. Key words: breast cancer, DDE, DDT, dieldrin, environmental estrogens, epidemiology, insecticides, mammmary cancer, organochlorines, pesticides. — Environ Health Perspect 109(suppl 1): 35-47 (2001).

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Address correspondence to S.M. Snedeker, Program on Breast Cancer and Environmental Risk Factors in New York State, 112 Rice Hall, Cornell University, Ithaca, NY 14853 USA. Telephone: (607) 254-2893. Fax: (607) 255-8207. E-mail: snms31@cornell.edu

The American Cancer Society has estimated that more than 182,800 new cases of breast cancer will be diagnosed in American women in the year 2000, and one in four of these women (40,800) will die of this disease (1). Because only about half of breast cancer risk can be attributed to established risk factors, including sex, advancing age, early menarche, late menopause, late age at first birth, and first-degree relative with breast cancer (2,3), there has been continued interest in the role environmental contaminants may play in unexplained breast cancer risk (4,5). Övarian hormones, including estrogen and progesterone, may affect breast cancer risk by affecting rates of cell proliferation in the breast or by supporting the growth of estrogen-dependent breast tumors (6–10). Hormonally active agents found in the environment that affect breast cell proliferation by acting as estrogen mimics or by disrupting pathways leading to enhanced breast cell proliferation may also affect breast cancer risk. In this review I discuss the results of human epidemiologic studies that have evaluated whether the persistent organochlorine insecticides dichlorodiphenyltrichloroethane (DDT) and dieldrin affect the incidence or mortality of breast cancer. DDT and DDE Over the last decade numerous epidemiologic investigations have been conducted to investigate whether environmentally persistent organochlorine pesticides affect the risk of breast cancer. The most widely studied pesticide has been DDT, an insecticide first used during World War II for control of lice and mosquitoes to combat typhus and malaria, respectively (11). DDT was used extensively in the United States for insect control in forestry and agriculture and for vector control until it was banned by the U.S. Environmental Protection Agency (U.S. EPA) in 1972 (12). Production of DDT reached its peak during the early 1960s at 81 million kg/yr (13). By 1966, although production had decreased, agricultural applications accounted for about 38% of the DDT used in the United States (14). By the early 1970s, use of DDT in the United States had declined dramatically to 4.5–6.4 million kg/yr, and its primary use was for pest control on cotton crops (14). However, agricultural regions were not the only areas with potential DDT contamination. It has been estimated that 2 million kg of DDT was expelled into the Los Angeles sewer system between 1949 and 1970 (15). Reproductive malformations in birds, including the feminization of male Channel Island gulls off the coast of southern California, have been attributed to DDT contamination (15–18). Forested areas were also sprayed extensively with DDT. During the 1980s and 1990s many Third World countries banned the use of DDT in agriculture, but use for vector control is still allowed. India banned the use of DDT in agriculture in 1989, although use against malaria-bearing mosquitoes is still prevalent (19).

The most prevalent breakdown product of DDT—dichlorodiphenyl dichloroethylene (DDE)—persists in the environment, concentrates up the food chain (20), is stored in fatty tissues of animals, fish, and humans (21–29), is widely detected in breast milk (30–34) and cow milk (35–37), and has been detected in household dust and air (38,39).

Why Evaluate DDT/DDE and Breast Cancer Risk?

Several lines of evidence supported investigating whether DDT or its metabolites affected breast cancer risk. These include the identification of some congeners of DDT as environmental estrogens (40–48). Technical DDT and o,p′-DDE, the most estrogenic component of technical DDT, can support the growth of estrogen-dependent breast tumors in rats, whereas metabolites of DDT that do not bind to the estrogen receptor (ER), such as dichlorodiphenyldichloroethylene (p,p′-DDE), are unable to support breast tumor growth (49). There is limited evidence that DDT may act as a promoter of mammary tumors in rats (50) and can inhibit gap junctional intercellular communication (51). Other evidence (52) of hormone-disrupting effects of DDT and its metabolites has included reproductive defects and eggshell thinning in avian species (16–18), sex reversal in medaka fish (52), and changes in sexual differentiation and behavior in mice (53). The persistent...
### Table 1. Human epidemiologic studies on DDE and breast cancer risk.

| First author (ref.) | Year(s) collected | Place (year published) | Cases/controls | Type of control | DDE concentration, mean (SD) | OR (95% CI) | p Trend | Association |
|---------------------|-------------------|------------------------|----------------|----------------|-------------------------------|--------------|---------|-------------|
| **Nested case–control studies, serum** | | | | | | | | |
| Knegtering (63) | 1996–1997 | California (1994) | 150/150 | Cases/controls | 43.3 (25.9) | 4.31 (23.7) ppb | 1.33 (0.68–2.2) | p = 0.43 | None |
| | | White | 50/50 | | 35.7 (23.0) | 3.50 (22.8) ppb | 2.38 (0.54–10.64) | p = 0.238 | None |
| | | Black | 50/50 | | 49.2 (28.6) | 4.34 (21.2) ppb | 3.85 (0.93–16.05) | p = 0.066 | None |
| | | Asian | 50/50 | | 45.1 (24.5) | 5.00 (24.7) ppb | 0.71 (0.23–2.18) | p = 0.516 | None |
| Hehl (73) | 1974 | Connecticut (2000) | 146/146 | | 11.5 (7.1) | 13.6 (10.6) ng/mL | 0.5 (0.27–0.89) | p = 0.02 | Negative |
| | | 1989 | 346/346 | | 7.9 (6.4) | 9.7 (3.8) ng/mL | 0.53 (0.24–1.17) | p = 0.08 | None |
| Luxembourg (73) | 1974 | Maryland (1999) | 346/346 | | 1.699 (929) | 1.920 (1,409) ng/g | 0.73 (0.40–1.32) | p = 0.13 | None |
| | | 1989 | 346/346 | | 1.311 (1,037) | 1.586 (1,557) ng/g | 0.58 (0.29–1.17) | p = 0.15 | None |
| Heyer (74) | 1976 | Denmark (1998) | 240/477 | | Not provided | 0.88 (0.56–1.37) | p = 0.52 | None |
| | | Dorgan (75) | 1977–1987 | | NA | 16.9 ng/mL | 0.8 (0.4–1.5) | p = 0.77 | None |
| | | Missouri (1999) | | | | | | |
| Wolf (77) | 1995–1996 | New York (1993) | 58/171 | | 11.8 (9.1) | 7.7 (6.8) ng/mL | 3.68 (1.01–13.50) | p = 0.035 | Positive |
| Wolf (64) | 1997–1998 | New York (2000) | 148/295 | | 6.95 (2.46) | 7.27 (2.39) ng/mL | 1.3 (0.51–3.35) | p = 0.99 | None |
| | | ER negative status | 32/64 | | 8.56 (1.87) | 6.42 (2.36) ng/mL | 1.83 (0.63–5.33) | p = 0.24 | None |
| | | ER positive status | 57/111 | | 7.04 (2.51) | 7.26 (2.21) ng/mL | 1.29 (0.54–3.05) | p = 0.44 | None |
| | | ER positive status | 23/42 | | 1.300 (1.79) | 1.040 (2.14) ng/g | 1.95 (1.10–3.52) | p = 0.02 | Positive |
| | | 1989–1990 | 232/236 | | 0.91 (0.56) | 0.97 (0.59) ppb | 0.72 (0.37–1.40) | p = 0.47 | None |
| Hunter (78) | 1997–1998 | Massachusetts (1997) | | | | | | |
| **Case–control studies, serum** | | | | | | | | |
| Moyer (78) | 1996–1997 | New York State (1998) | 154/192 | | 11.47 (10.49) | 10.77 (10.64) ng/g | 1.34 (0.71–2.55) | p = 0.25 | None |
| | | Never lactated | 46/61 | | 13.15 (11.65) | 10.82 (10.91) ng/g | 1.83 (0.63–5.33) | p = 0.24 | None |
| | | Ever lactated | 85/106 | | 10.36 (8.97) | 10.44 (10.43) ng/g | 1.29 (0.54–3.05) | p = 0.44 | None |
| Romieu (80) | 1990–1994 | Mexico (2000) | 120/126 | | 3.84 (5.98) | 2.52 (1.97) ng/g | 3.81 (1.14–12.80) | p = 0.02 | Positive |
| | | | | | (lipid basis) | | | |
| | | | | | 24.2 | 17.5 µg/L | | | |
| Scharfenberg (79) | 1994 | North Vietnam (1997) | 20/20 | | 12.17 (2.41) | 16.67 (4.14) ng/mL | 1.14 (0.23–5.88) | p = 0.47 | None |
| | | Lopez-Camacho (80) | 1994–1996 | | 141/141 | 562.5 (676.2) | 505.5 (567.2) ppb | 0.76 (0.41–1.42) | p = 0.14 | None |
| | | Mexico (1997) | | | | | | |
| Demers (81) | 1994–1997 | Quebec, Canada (2000) | 314/218 | | 508.9 (491.1) | 462.7 (447.7) µg/kg | 1.36 (0.71–2.63) | p = 0.82 | None |
| | | Olaya-Contreras (82) | 1995–1996 | | 563/305 | 480.4 (408.1) µg/kg | 1.00 (0.40–1.67) | p = 0.14 | None |
| | | Colombia, So. Am. (1998) | | | 3.30 (4.12) | 2.50 (3.80) ng/mL | 1.95 (1.10–3.52) | p = 0.09 | None |
| | | Mandouca (83) | 1995–1996 | | 151/306 | 3.1 | 4.8 ng/mL | 0.83 (0.40–1.6) | p = 0.79 | None |
| | | Brazil (1999) | | | | | | |
| Zheng (84) | 1995–1997 | Connecticut (2000) | 475/502 | | 460.1 | 456.2 ppb | 0.96 (0.67–1.36) | p = 0.58 | None |
| | | Cases, ER negative status | 13/36 | | 343.1 ppb | Not reported | | | |
| | | Cases, ER positive status | 140/452 | | 343.9 ppb | Not reported | | | |
| Dello Iacovo (86) | 1997–1998 | Italy (1999) | 170/195 | | 9.55 (5.42) | 8.96 (5.17) ng/mL | | | |
| | | Descriptive and case–control studies, adipose tissue | | | | | | |
| Unger (88) | 1995–1997 | Early 1980s (surgery for noncancer breast diseases) | 14/27 | | 1.23 (0.63) | 1.25 (0.76) ppm | | | |
| Falck (72) | 1987 | Connecticut (1992) | 20/20 | | 2.200 (1,470) | 1.487 (842) ng/g | | | |
| (Continued) | | | | | | | | |
metabolite \( p,p' \)-DDE and to a lesser extent the DDT isomer \( p,p' \)-DDT are still detected in low amounts in food, especially in fish, meat and dairy products, and root vegetables (26,35,54–59). DDT and DDE are stored in human adipose tissue, and levels increase as a function of age (21,62–65). However, the levels of DDE detected in food (26,62) and in blood and adipose tissues of humans in Western countries (21,62–65) have been decreasing since the decline of DDT use during the 1960s. Even though DDT sales and use were banned in the United States and Canada in the early 1970s, new deposits of DDT are still detected in North America through aerial deposition from other countries that continue to use DDT (66). The continuation of new inputs via atmospheric deposition in the Arctic Circle is supported by the relatively high body burdens of DDE reported in native populations of Greenland (67).

**Overview of the Epidemiologic Studies on DDT, DDE, and Breast Cancer Risk**

One of the first studies that explored a possible relationship between tissue levels of DDE and cancer risk in women was conducted by Unger and Olsen (68). Levels of DDE in adipose tissue increased as a function of age in both men and women with and without cancer. Mean levels of DDE extracted from abdominal adipose tissue were higher in a group of 11 women with cancer (5.03 parts per million (ppm)) compared to 22 women without cancer (2.14 ppm). In a small follow-up study, breast adipose tissue biopsy samples were obtained from 14 women with breast cancer and 21 women with other noncancerous breast diseases (69). There was no relationship between mean levels of DDE in women with breast cancer (1.23 ppm) compared to those in controls (1.25 ppm). Several other small case–control studies conducted in the early 1990s compared adipose tissue levels of DDE in women with breast cancer to controls without breast cancer (70–72). These studies found a positive relationship between DDE levels and breast cancer risk (Table 1); however, interpreting the significance of these results and the studies by Unger et al. is difficult because of their very small sample size (<25 cases); use of controls with benign or other types of breast disease, and lack of control for potential confounding factors that could affect breast cancer risk.

These early descriptive and case–control studies stimulated interest in investigating the relationship between DDT and DDE and breast cancer risk, and led to more carefully designed nested case–control studies (63,64, 73–77) and case–control studies (78–92) that are summarized in Table 1. These studies considered the effects of various confounding factors on the relationship between tissue DDE levels and breast cancer risk. Mussalo-Rauhamaa et al. (91) compared adipose tissue levels of \( p,p' \)-DDT and \( p,p' \)-DDE from 44 women with breast cancer to levels in postmortem tissue samples from 33 women who had died accidental deaths. There were no significant differences between mean levels of DDE or DDT between cases (\( p,p' \)-DDT 0.07 \pm 0.09 mg/kg fat; \( p,p' \)-DDE 0.96 \pm 0.63 mg/kg fat) and controls (\( p,p' \)-DDT 0.06 \pm 0.07 mg/kg fat; \( p,p' \)-DDE 0.98 \pm 0.89 mg/kg fat), respectively.

Two prospective nested case–control studies with very different conclusions were published in the early 1990s. Wolff et al. (77) obtained blood samples between 1985 and 1991 from 14,290 women enrolled in the New York University Women’s Health Study. The cohort members who developed breast cancer (\( n = 58 \)) and controls (\( n = 171 \)) were matched for age at entry into the study,

### Table 1. Continued.

| First author (ref.) | Year(s) collected sample | Cases/controls | Type of control | DDE concentration, mean (SD) | OR (95% CI)* | \( p \) Trend | Association |
|---------------------|--------------------------|---------------|----------------|------------------------------|--------------|-------------|------------|
| Mussalo-Rauhamaa et al. (91) | 1996–1998 | 44/33 | PM | 0.96 (0.53) | 0.98 (0.89) mg/kg | None | |
| Dewailly et al. (70) | 1991–1992 | /7 | BBD | 765.3 (526.9) \( \mu g/kg \) | | | |
| Cases, ER negative | 9/ | | | | | | |
| Cases, ER positive | 9/ | | | | | | |
| Djordjevic et al. (77) | Early 1990s | 5/5 | HC | 379 (286) | 160 (149) ppb | Positive | |
| Güttes et al. (86) | 1993–1994 | 45/20 | BBD | 805 | 496 \( \mu g/kg \) | Positive | |
| Germany (98) | | | | | | | |
| Ljiljegren et al. (67) | 1993–1995 | 35/43 | BBD | 767 | 1.026 ng/g | 0.4 (0.1–1.2) Negative | |
| Sweden (98) | | | | | | | |
| Zheng et al. (62) | 1994–1997 | 304/186 | BBD | 736.5 | 736.5 ppb | 0.9 (0.5–1.5) \( p = 0.46 \) None | |
| Connecticut (99) | | | | | | | |
| van’t Veer et al. (88) | 1996 | 347/374 | HC, CC | 1.35 (median) | 1.51 \( \mu g/g \) | 0.48 (0.25–0.95) None | |
| Europe (1997) | | | | | | | |
| Aronson et al. (89) | Late 1990s | 217/213 | BBD | 693 | 596 \( \mu g/kg \) | 1.62 (0.84–3.11) None | |

**Mortality correlation study, adipose tissue**

| Place (year published) | Year collected sample | Correlation between adipose tissue and breast cancer mortality | Association |
|------------------------|-----------------------|-------------------------------------------------------------|-------------|
| Cocco (88) | 1968 | | |
| United States (2000) | | \(-0.731^{**}\) Negative | |
| Black women | | \(-0.501^*\) Negative | |

Abbreviations: BBD, benign breast disease controls; HC, hospital controls (hospitalized for nonbreast disease and noncancer related conditions unless otherwise noted); CC, community or population controls; PM, postmortem autopsy controls; ref., reference number; SD, standard deviation; So. Am., South America.

*OR adjusted for potential confounding factors, comparison between highest and lowest levels of DDE (i.e., tertile, quartile, or quintile). **Serum DDE adjusted for lipid concentration. *Plasma DDE adjusted for cholesterol.

\* \( p < 0.05 \), \* \( p < 0.01 \).
menopausal status, and dates of blood donations. The relative risk of developing breast cancer was nearly 4-fold higher in women with the highest levels of serum DDE (OR 3.68, 95% confidence interval (CI) 1.01–13.50, p trend = 0.035; highest quintile compared to lowest quintile). Relative risks were adjusted for first-degree family history of breast cancer, lactation, and age at first full-term pregnancy.

These findings were in contrast to conclusions drawn from the results of a nested case–control study conducted in northern California (63). Subjects were from a cohort of 57,040 women who donated blood samples from 1964 to 1971, a period when DDT was still in use in the United States. The mean time between obtaining the blood samples and the diagnosis of breast cancer was 14.2 years. The serum DDE levels were compared in 150 breast cancer cases to 150 matched controls. There were no statistically significant differences in the relative risk of developing breast cancer between women in the highest tercile for serum DDE levels compared to women in the lowest tercile [OR 1.33, 95% CI 0.68–2.2; OR adjusted for body mass index (BMI), age at menarche, ever vs. never pregnant, menopausal status, year of examination, and length of follow-up time]. Nor were there significant differences within white, Asian, or black subgroups (50 cases and controls in each subgroup), although serum DDE levels tended to be higher among black cases than controls (Table 1). The significance of the results from the different racial/ethnic subgroups is discussed more fully later in this review.

From 1997 to June 2000, five more nested case–control studies were published comparing levels of DDE in serum of women with and without breast cancer. This includes studies conducted in American women in Maryland by Helzlsouer et al. (73) and in Missouri by Dorgan et al. (74), a follow-up of the New York University Women’s Health Study by Wolff et al. (64), a nested case–control study drawn from the Harvard Nurses’ Health Study cohort by Hunter et al. (76), and a study of Danish women enrolled in the Copenhagen City Heart Study by Huyer et al. (74). The results of these studies are summarized in Table 1 in chronologic order according to when the blood samples were obtained.

None of these studies supported the findings of the 1993 study by Wolff et al. (77) that a strong positive relationship exists between breast cancer risk and serum DDE levels. Wolff et al. (64), in a subsequent follow-up study that included 148 breast cancer cases and 295 matched controls, did not confirm their previous observation of increased breast cancer risk with higher body burdens of DDE. Instead they reported no association of DDE serum levels and breast cancer risk (OR 1.33, 95% CI 0.51–3.35, p trend = 0.99) even when the relative risk was adjusted for potential confounders such as age at menarche, number of full-term pregnancies, family history of breast cancer, lactation history, height, BMI, and menopausal status. The four other cohort studies published since 1998 reported relative risks below 1, even after adjusting for confounders (73–76).

Similarly, most of the well-controlled case–control studies published since 1998 failed to find a significant positive relationship between breast cancer risk and the levels of DDE or DDT in blood (78–81,83–85) or in adipose tissue (87–89,91,92). Although some studies have reported relative risks > 1.1 (78,79,81,89) or < 0.9 (80,83,87), none of these studies had ORs that reached statistical significance, and this supports a lack of association. One of the few studies that has reported a positive relationship between serum DDE levels and breast cancer risk was conducted in 153 women with breast cancer and 153 age-matched controls from Colombia, South America (82). Plasma DDE levels were significantly higher (p < 0.025) in the subjects with breast cancer (3.30 ± 4.12 ng/mL) than those in hospital controls without breast cancer (2.50 ± 3.60 ng/mL). However, the relative risks were at the borderline of statistical significance when upper and lower terciles were compared, even though the confidence interval did not embrace 1 (OR 1.95, 95% CI 1.10–3.2, p trend = 0.09).

Use of DDT in Colombia, although lower than in many other South American countries, is more recent than use in the United States. A case–control study of 120 women with breast cancer and 126 age-matched community controls from Mexico City, Mexico, also found a positive association between serum p,p′-DDE levels and breast cancer risk (OR 3.81, 95% CI 1.14–12.80, p trend = 0.02), whereas serum p,p′-DDT levels were not related to breast cancer risk (90). As I discuss later in this review, in contrast, other studies conducted in countries with current or recent DDT use have not confirmed a relationship between DDE exposure and breast cancer risk (79,80,83).

The only investigation that has reported a positive relationship between tissue levels of DDE and breast cancer risk in a Western country was a case–control study conducted in Germany (86). A significantly higher (p = 0.017) mean level of p,p′-DDE was found in the breast tumor tissue of 45 women with breast cancer (805 µg/kg) compared to breast tissue obtained from 20 women with benign breast disease (BBD) (496 µg/kg). However, interpretation of this study is difficult because instead of comparing a sample of breast tissue adjacent to the tumor, the levels of DDE in the tumor tissue itself were compared to DDE levels in the breast tissue of the controls who had BBD.

**Differences in Analytic Methodologies**

There have been some discussions among researchers of the advantages and disadvantages of using serum versus adipose tissue levels to determine body burdens of organochlorine compounds, including DDE or DDT. The half-life of DDE in humans has recently been estimated at 13 years (64). Because of its persistence and slow elimination from the body, DDE is the most prevalent form of DDT detected in human tissues. Adipose tissue samples have the advantage of having higher values for organochlorines than blood, and breast adipose samples predict the level of organochlorines to which the breast is exposed over time (89). However, because obtaining adipose tissue samples is highly invasive, this usually limits the availability of the control population that can be recruited, and most of the adipose tissue case–control studies conducted have used women with BBD or women being admitted for a breast biopsy sample as controls. Several recent studies have shown good agreement between adipose tissue and blood levels of DDE (22,93,94). Hence, there is evidence that blood levels of DDE are a good approximation of the DDE stored in adipose tissue.

One study has examined the relationship between the ratio of adipose DDE levels and serum DDE levels. The ratio was nearest to a value of 1 when serum DDE levels were expressed as the geometric mean on a lipid basis, and did not approach 1 when expressed as the arithmetic mean or on a wet weight basis (94). To achieve normality, serum DDE levels are often converted to another scale (log, square root) before statistical comparisons are made. However, it is desirable to have the arithmetic means to compare results of different studies.

Some studies have expressed blood DDE levels on a volume or wet weight basis (63,73,75,77,79,80,82,83,85), whereas others have corrected blood DDE levels for lipid (64,73,78,80,81,84,90) or cholesterol content (76). Because DDE is carried in the lipid fraction of the blood, expressing serum DDE levels adjusted for lipid content is generally considered a more desirable method for reporting serum DDE levels. Because serum lipid levels rise with fasting, this is an especially important correction when blood samples were obtained in a fasting state. The units used to express serum DDE levels affected the significance of the results in one study. Helzlsouer et al. (73) found a significant negative relationship when ORs were calculated based on serum DDE levels.
expressed on a volume basis as nanograms per milliliter (OR 0.5, p trend = 0.02), but there was not a statistically significant negative relationship when ORs were calculated based on serum DDE levels expressed per gram of lipid (OR 0.73, p trend = 0.13) (Table 1). Lopez-Carrillo et al. (80) and Wolff et al. (64) did not find an association of serum DDE levels with breast cancer risk regardless of whether the results were expressed on a volume or a lipid-corrected basis. As mentioned previously, in an earlier study conducted with the same cohort Wolff et al. (77) found a strong positive relationship between serum DDE levels and breast cancer risk. Although the serum DDE levels in this study were expressed on a per-volume basis, it is unlikely that a correction for lipid levels would explain the magnitude of the difference between the mean serum DDE levels in cases (11.8 ± 9.1 µg/mL) and controls (7.7 ± 6.8 µg/mL). Romieu et al. (90) reported a significantly higher (p < 0.05) mean serum p,p’-DDE level in women with breast cancer (24.2 ± 13.8 µg/g lipid) compared to community controls without breast cancer (17.5 ± 5.6 µg/g lipid) when results were expressed on a volume basis or when corrected for lipids, respectively. A lack of an association of serum DDE levels and breast cancer risk has been reported in both studies that have (64,73,74,76,78,80,81,84) and have not (63,75,79,83,85) corrected for the lipid or cholesterol content of serum. Therefore, there does not appear to be a tendency to report positive relationships between serum DDE levels and breast cancer risk in the studies that have not adjusted serum DDE levels for lipid content.

Differences in analytic methodology, quality control (QC), and quality assurance (QA) programs may affect the magnitude of tissue DDE levels reported in different laboratories. It is beyond the scope of this review to give an in-depth review of the analytic control procedures used in each study; however, this section provides an overview of the procedures used by different laboratories. Most investigators reported using an analytic procedure to determine DDE in serum/tissue that included extracting samples with organic solvents to obtain the lipid fraction, concentration of the sample, purification using Florisil columns, and identification and quantification of the DDE in the sample by high-resolution gas chromatography–low-resolution mass spectrometry followed by GC analysis (71). The use of U.S. EPA-approved methods has also been cited by other investigators (80,82).

The use of QA and QC procedures is important with any analytic procedure. Several studies, especially some of the earlier studies, did not report whether QA or QC procedures were used (68–70,87). Several investigators reported participating in regional programs with interlaboratory QA/QC protocols (78,86,89–91). Other controls included running analyzates in small batches with samples from cases, controls, reagent blanks and/or QC pooled samples (64,73,74,76,78,84,89,92) and/or blinding laboratory personnel to the nature of the sample (78,88,89). The coefficient of variation in pooled control samples run with each batch differed widely among different laboratories. Coefficients of variation <10% were reported by several laboratories (73,76,88,92), whereas a coefficient of variation of 21% was reported by Dorgán et al. (75). The percent recovery of DDE from spiked samples also varied from laboratory to laboratory. Zheng et al. (92) reported recoveries exceeding 95%; Falck et al. (72) reported recoveries ranging from 90 to 109%; and Musso-Raahama et al. (91) reported an 80% recovery of an internal standard.

Relative Estrogenic Potencies of DDT and DDE

The lack of a positive relationship between levels of DDE in blood or adipose tissue and breast cancer in many of the published studies may be caused by the route and magnitude of exposure to the various congeners of DDT and metabolites that persist in the environment and by the differences in their estrogenicity. The technical DDT that was sprayed as an insecticide was not a single chemical but was actually a complex mixture of several DDT congeners (19). Although the most estrogenic component of technical DDT has been consistently identified as p,p’-DDE, this form of DDT comprised only about 15–23% by weight of the technical DDT. About 77% of the technical DDT was composed of another congener, p,p’-DDT. Through the loss of a chlorine, DDT can degrade to DDE, which has little to no evidence of estrogenicity. Although there is some deposition of DDT onto American and Canadian soils via volatilization and atmospheric transport from

**Pesticides and breast cancer risk**

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countries in Central and South America that still use DDT, the magnitude of exposure through this route is considered to be small (68). Because of widespread past use of DDT and its persistence and slow degradation of its metabolite DDE, low levels of p,p'-DDE residues are still detected frequently in foods consumed by Americans, especially in animal and dairy products, fish, root vegetables, and legumes (20,25,26,35,55,58,104). In the U.S. Food and Drug Administration (FDA) market-basket surveys conducted from 1991 to 1997, p,p'-DDT and o,p'-DDT were detected far less frequently than residues of p,p'-DDE (59).

Part of the explanation for why most North American and European studies have failed to find an association between blood or adipose tissue levels of DDE and breast cancer risk may be that since the 1970s the major route of exposure to DDT has not been through the more estrogenic o,p'-DDT found in technical DDT that was sprayed as an insecticide but through the far less estrogenic p,p'-DDE via the diet. Although use of DDT in the United States was banned in 1972, DDT production peaked much earlier, in 1963 at 81 million kg/yr; about 36 million kg/yr was used in the United States and the rest was exported. By 1968, use of DDT in the United States had declined to approximately 15 million kg/yr (18). The only epidemiologic study that collected serum samples from women when DDT was still being used heavily in the United States was conducted by Krieger et al. (63). Samples were collected from 1964 to 1971. This study did not find a strong positive association between DDE levels in serum and breast cancer risk (Table 1).

Unfortunately, despite the extensive use of DDT in agriculture, especially on cotton crops in the late 1960s, none of the American epidemiologic studies have evaluated breast cancer risk in rural farm women potentially exposed to DDT while it was being used to treat crops. Other investigators have conducted studies in countries where DDT has been used more recently and hence there is a greater potential for women to be exposed to technical DDT. A small pilot case–control study compared the serum levels of p,p'-DDT and p,p'-DDE in 21 women with breast cancer to 21 controls with BBD from North Vietnam (79). The primary use of DDT in Vietnam is for mosquito control. There was no increase in the relative risk of breast cancer with higher levels of serum DDE or DDT in this study, even after adjustment for age at menarche, parity, history of lactation, and body weight (DDE OR 1.14, 95% CI 0.23–5.68; DDT OR 1.21, 95% CI 0.15–9.65; highest tercile compared to lowest tercile). This study did observe a 3-fold higher level of serum DDT and DDE in urban populations compared to rural residents. The authors suggested that urban residents who could afford to buy potentially contaminated animal products may be ingesting more DDT/DDE than are less economically advantaged rural farmers who produce the food.

Although the use of DDT in Mexico has declined from 8,000 tons in 1971 to 2,000 tons in 1994, DDT is still used to control mosquitoes. A case–control study was conducted to compare serum DDE and DDT levels in 141 women with breast cancer to 141 noncancer hospital controls from Mexico City (80). Mean serum DDE levels were slightly, but not significantly, higher in cases (502.5 parts per billion (ppb), lipid adjusted) compared to controls (505.5 ppb, p = 0.44), and the age-adjusted OR for breast cancer was not different when the lowest tercile for serum DDE was compared with the highest tercile (OR 0.97, 95% CI 0.55–1.70). The OR was lowered even further when adjusted for BMI, breast feeding, parity, family history of breast cancer, and time elapsed since last birth (OR 0.76, 95% CI 0.41–1.42). Relative risks were not calculated for serum levels of DDT. Unlike those of p,p'-DDE, the mean serum levels of p,p'-DDT were lower in cases (61.45 ppb) than in controls (84.53 ppb), but this effect was not significant (p = 0.26). Even though DDT is still used in Mexico for vector control, the valley where the subjects resided was not an area sprayed for mosquitoes. It is possible that the route of exposure to DDT in urban areas such as Mexico City may be primarily through DDT in the diet and may not be appreciably different from exposure patterns in other Western countries.

In contrast, a more recent study conducted in Mexico City found significantly higher serum levels of p,p'-DDE in women with breast cancer than in women without breast cancer (90). In this study of 120 breast cancer cases and 126 age-matched community controls, mean serum p,p'-DDE levels (cases 3.84 µg/g lipid, controls 2.51 µg/g lipid) but not serum p,p'-DDT levels (cases 0.15 µg/g lipid, controls 0.23 µg/g lipid) were significantly elevated (p < 0.05) in women with breast cancer compared to women without the disease. The reason for the different results in these two studies with subjects from Mexico City is not clear. Romieu et al. (90) in their discussion, mention that the magnitudes of the serum DDE levels are much higher in their study compared to the values reported by Lopez-Carrillo et al. (80). If the serum DDE values are converted to the same units, then the mean serum DDE levels per gram lipid are about 7-fold higher in cases (3.84 µg/g vs. 0.56 µg/g) and 5-fold higher in controls (2.52 µg/g vs. 0.51 µg/g) in the Romieu et al. study compared to the Lopez-Carrillo study, respectively. However, why the magnitude of the serum DDE levels would be so different in a population recruited from the same metropolitan area is not apparent. The study populations did have different characteristics. All the subjects in the Romieu et al. study were parous (at least one pregnancy), whereas nulliparity was associated with a higher breast cancer risk among cases in the Lopez-Carrillo et al. study. However, one would expect that subjects who were not parous and therefore had not lactated would have a higher body burden of DDE. Another possible explanation would be differences in the analytic methods or QA/QC procedures between the two laboratories. Both studies appear to have used appropriate methods to analyze the samples. Lopez-Carrillo et al. (80) used a U.S. EPA-approved method to analyze samples, but details on the QA and QC procedures used were not provided. Romieu et al. (90) used a standard solvent extraction, Florisil purification, and gas chromatography with electron capture detection procedure. QC procedures included running internal controls with each batch of samples, and accuracy was verified by interlaboratory comparisons.

No relationship between DDE levels and breast cancer risk was observed in a Brazilian case–control study of 177 breast cancer cases and 350 community controls recruited from female visitors at the hospital (OR 0.83, 95% CI 0.40–1.6, p trend = 0.79; comparing highest to lowest quintile, OR adjusted for age, education, parity, lactation, tobacco smoking, family history of breast cancer, and breast size) (83). The authors noted that organochlorine pesticides were used in Brazil for agriculture until the 1980s and for vector control programs until the 1990s. This was one of the few studies that attempted to obtain information on occupational and household use of pesticides, including time lived in a rural area, time lived in a rural area with pesticide use, time lived in an area with a vector control program, and occupational exposure. No relationship was observed between these types of potential exposures and differences in breast cancer risk between cases and controls.

Serum levels of DDE in 153 breast cancer cases and 153 age-matched controls who were noncancer hospital patients were compared in a case–control study conducted in Colombia, South America (82). The relative risk of breast cancer was approximately 2-fold higher in women in the highest tercile compared to those in the lowest tercile (OR 1.95, 95% CI 1.10–3.52; OR adjusted for breastfeeding, first child, familial history of breast cancer, parity, BMI, and menopausal status), although test for trend indicated borderline
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Statistical significance ($p$ trend = 0.09). DDT was used until 1986 in Colombian agriculture primarily for cotton, rice, and flower production and until 1994 for vector control. One explanation given for the differences between the positive relationship between serum DDE and breast cancer risk in this study (82) versus the lack of relationship in Lopez-Carrillo et al.’s (80) Mexican study was differences in the diet between Mexico and Colombia. Olaya-Contreras et al. (82) cited a great availability of a variety of fruits, vegetables, seeds, and grains in Mexico compared to urban areas of Colombia. No actual dietary data were collected or reported in either study, so there are no data to support this hypothesis.

One shortcoming of most of the studies that evaluated the relationship between DDT, DDE, and breast cancer risk is the lack of extensive exposure histories. Seldom have the studies attempted to determine whether the individuals enrolled in the study lived in areas sprayed with DDT, nor have dietary histories been taken to estimate possible foodborne exposures. Although serum or tissue levels of DDE serve as biomarkers of past exposure to all forms of DDT, it would strengthen studies to also gather information on potential past routes of exposure. Although recall bias is a common problem in such surveys, especially among cancer patients, exposure histories might help interpret study results. The number of published studies conducted in countries currently using DDT is relatively low, and none could be located from countries where there is still very heavy DDT spraying, such as India. If the most estrogenic component of technical DDT—$\pi,p’\text{-DDT}$—has the potential to act as an environmental estrogen and to promote the growth of estrogen-dependent tumors, women in countries with long and current histories of DDT use would be the most important to study if we are to understand the relationship between DDT and breast cancer risk. Another potential population to monitor would be women in the U.S. armed forces or female spouses of military personnel who have been stationed in countries that have actively used DDT during their terms of service.

Dietary Factors

Few studies evaluating breast cancer risk and organochlorine levels have collected dietary data on their subjects. Moysich et al. (78) found no differences in postmenopausal breast cancer patients and community controls with regard to their fruit, vegetable, dairy, fish, or meat consumption. One exception was found when women were characterized as those who had ever and those who had never lactated. Vegetable consumption was significantly higher in ever-lactating controls compared to breast cancer cases, though no dietary differences were observed between cases and controls in the never-lactated group. There were no significant associations between serum DDE and breast cancer risk in this study, Demers et al. (81), in a case–control study conducted in New Haven, Connecticut, did find that control subjects tended to have diets with lower fat intakes than did breast cancer cases, and fat intake was one of the confounders used to adjust the relative risks in this study. However, they found no relationship between serum DDE levels and breast cancer risk. Aronson et al. (89), in a case–control study of Canadian women, also reported a higher intake of dietary fat in cases than in hospital controls with BBD. Dietary fat was one of the covariates included in the confounder model, and although breast cancer risk was moderately elevated in women with the highest breast adipose DDE levels (OR 1.62, 95% CI 0.84–3.11), this effect was not statistically significant.

Relationship of DDE to Menopausal Status and Breast Cancer Risk

Several studies have calculated relative risks of breast cancer in relation to tissue levels of DDE after stratifying their sample by menopausal status. In the study of Canadian women by Aronson et al., the relative risk of breast cancer was moderately, though not significantly, higher in premenopausal women (OR 1.52, 95% CI 0.7–3.33). In contrast, there was no association in the postmenopausal women (OR 1.05, 95% CI 0.5–3.33) (89). A Colombian study also reported relative risks stratified by menopausal status. The relative risk was higher in premenopausal women (OR 2.46, 95% CI 0.96–6.30) than in postmenopausal women (OR 1.85, 95%, CI 0.85–4.05), although both groups had elevated relative risks that were not statistically significant (82). This is in contrast to the cohort study of Helzlsouer et al. (79), which evaluated breast cancer risk in relation to serum DDE levels in two cohorts: one that donated blood in 1974 and one that donated blood in 1989. They did not identify any pattern in regard to menopausal status and breast cancer risk in women with elevated lipid-adjusted serum DDE levels compared to tercile. Relative risk was decreased in both postmenopausal (OR 0.52) and premenopausal women (OR 0.86) in the 1974 cohort, whereas the relative risk was elevated in premenopausal women (OR 1.42) but remained below 1 in the postmenopausal women (OR 0.50) in the 1989 cohort. A small case–control study of only premenopausal Vietnamese women found no association between serum DDE levels and breast cancer risk (OR 1.23, 95% CI 0.23–5.68), and serum DDE levels were not associated with an increased risk of breast cancer in a case–control study of postmenopausal women from western New York (78). Lopez-Carrillo et al. (80), in a case–control study of women from Mexico City, did not observe elevated breast cancer risk associated with serum DDE levels in premenopausal women (OR 0.64, 95% CI 0.22–1.90) or in postmenopausal women (OR 0.79, 95% CI 0.27–2.28). In contrast, Romieu et al. (90) reported a significant association between elevated serum DDE levels and risk of breast cancer in postmenopausal women from Mexico City (OR 5.26, 95% CI 0.80–34.30, $p$ trend = 0.03; OR adjusted for age, age at menarche, duration of lactation, BMI, and serum DDE levels adjusted for lipid). Breast cancer risk was not associated with serum DDE levels in the premenopausal women in this study (OR 2.41, 95% CI 0.37–15.81, $p$ trend = 0.16). There does not appear to be a consistent pattern of breast cancer risk related to body levels of DDE and menopausal status.

Different Types of Control Populations

A potential confounding factor in many of the case–control studies investigating serum or adipose tissue organochlorine pesticides levels and breast cancer risk is the use of controls with BBD. There is some evidence that women with a previous history of BBD have a higher risk of breast cancer (76,105). Of the larger, well-controlled case–control studies, six recruited controls with BBD (79,84,86,87,89,93). Except for the study by Güttes et al. (80), none found a significant positive relationship between levels of DDE and breast cancer risk. (I previously discussed the limitations of the study by Güttes et al.).

The results of most of the case–control studies that used other types of control populations also did not support the hypothesis that high tissue or blood levels of DDE are positively related to breast cancer risk. This includes the study by Lopez-Carrillo et al. (80) that used noncancer hospitalized controls, the case–control study conducted by Demers et al. (81) that used hospitalized noncancer surgical controls, and the European study conducted by van’t Veer (88) that used a combination of population and hospital-based controls (underlying condition not specified). However, all of these studies used at least some controls that had underlying medical conditions. One case–control study that reported a significantly higher level of blood DDE in breast cancer patients compared to controls did use noncancer hospital-based controls and was conducted in Colombia, a country that has a history of recent use of DDT in agriculture and vector control (82).
Four case–control studies \(^{(78,83,85,90)}\) used community/population-based controls, and three of the studies \((73,78,80)\) did not report an association between DDE and breast cancer. This includes a Brazilian case–control study that compared serum DDE levels of 151 breast cancer patients with those of 306 community controls recruited from hospital visitors, Mendonca et al. \((83)\) did not observe a significant difference between serum DDE levels in controls (4.8 ng/mL) compared to those in women with breast cancer (3.1 ng/mL, \(p = 0.93\)). A case–control study conducted in Italy compared serum DDE levels in 170 breast cancer cases and those in 190 community controls \((85)\). The community controls were healthy women participating in a study on diet and cancer. Serum \(p,p^\prime\)-DDE levels were similar in cases (9.55 ± 5.42 ng/mL) and controls (8.98 ± 5.17 ng/mL), and relative risks were not significantly elevated when the highest to the lowest tertiles were compared, even when adjusted for age, BMI, lactation, parity, and serum lipids \((OR = 1.24, 95\% CI 0.70–2.20)\).

One study that recruited controls from the community was conducted by Moysich et al. \((78)\). This study of western New York women included 154 postmenopausal women with histologically confirmed breast cancer and 192 postmenopausal community controls recruited using names from Health Care Finance Administration and New York State Department of Motor Vehicle records. Mean levels of serum DDE adjusted for age and serum lipids were similar in cases (11.47 ± 10.49 ng/mL) and controls (10.77 ± 10.64 ng/mL). Although the risk of breast cancer was moderately elevated in women in the highest tercile compared to the lowest tercile for serum DDE, this effect was not statistically significant \((OR = 1.34, 95\% CI 0.71–2.55, p = 0.25)\); OR adjusted for age, education, family history of breast cancer, parity, BMI index, duration of lactation, age at first birth, years since last pregnancy, fruit and vegetable intake, and serum lipids). The only study using community controls that did find a significant positive relationship between serum DDE levels and breast cancer risk was conducted by Romieu et al. \((90)\), and included 120 breast cancer cases and 126 community controls from Mexico City. Cases were recruited from six major hospitals, whereas controls were randomly selected from the Mexico City metropolitan area using the National Household Sampling Frame. The mean levels of serum \(p,p^\prime\)-DDE were significantly higher in breast cancer cases (3.84 µg/g lipid, \(p = 0.05\) by t-test) than in controls (2.51 µg/g lipid).

There do not appear to be differences in the relationship between tissue DDE levels and breast cancer risk regardless of the type of control population used. Most of the studies using either BBD controls, hospital controls, or community controls did not find a relationship between levels of DDE or DDT and breast cancer risk.

### Lactation History

The study of western New York women by Moysich et al. \((78)\) is the first to stratify cases and controls by lactation history, classifying mammograms as with breast Feeney in 1980 case–control study that compared serum DDE levels in 170 breast cancer cases and 190 community controls \((85)\). When comparing the highest to the lowest tertiles, the risk of breast cancer was higher, but not significantly higher, for those who had never lactated \((OR = 1.83, 95\% CI 0.63-5.33, p = 0.24)\), whereas there was no association between serum DDE levels and breast cancer risk among those who had ever lactated \((OR = 1.28, 95\% CI 0.54-3.05, p = 0.44)\). Aronson et al. \((89)\) also stratified their study by lactation history but found an association between lactation and breast cancer risk only for another organochlorine, Mirex, and not for adipose DDE. Several case–control and cohort studies did consider lactation history as a potential confounder \((64,73,76,77,81–84,90,92)\). Of the studies that controlled for lactation in their covariate model, the two studies that found an association between breast cancer risk and DDE levels were the 1993 study conducted by Wolff and colleagues \((64)\), and the study of women from Mexico City conducted by Romieu et al. \((90)\).

### Estrogen Receptor Status

Researchers have also investigated whether ER status of breast tumors is related to body burdens of DDT or DDE. Dewailly et al. \((70)\) compared the concentrations of DDE in the breast adipose tissue of 9 women with ER-negative breast tumors, 9 women with ER-positive tumors, and 17 controls with BBD. The mean concentrations of DDE in breast adipose tissue were substantially higher in the women with ER-positive breast tumors \((2.132.2 ± 2.049 \mu g/kg)\) compared with levels in women with ER-negative breast tumors \((608 ± 338.9 \mu g/kg)\) or controls \((765.3 ± 526.9 \mu g/kg)\). Other case–control and nested case–control studies did not find a relationship between ER-positive status and levels of DDE in breast \((64,73,76,84)\) or adipose tissue \((87,88)\). For example, Zheng et al. \((94)\) reported very similar mean serum DDE levels for the 163 cases with ER-positive tumors \((435.5 \mu g/kg)\) and the 140 cases with ER-negative tumors \((453.9 \mu g/kg)\). In a study conducted by Wolff and colleagues \((64)\), the geometric mean serum DDE levels were not higher, but were lower in cases with ER-positive tumors \((950 ± 2.41 ng/mL)\) compared to cases with ER-negative breast tumors \((1,300 ± 1.79 ng/mL)\). Studies published to date have not confirmed the observation originally made by Dewailly et al. \((70)\) of a relationship between ER-positive receptor status of breast tumors and tissue levels of DDE.

### Ethnic/Racial Subgroups

Survey studies conducted in the 1970s and 1980s consistently demonstrated that adipose tissue levels of DDE were higher in American blacks than in whites \((28,106)\), although whether higher body burdens of DDE affected the risk of breast cancer was not evaluated in these studies. In 1994 Krieger et al. \((63)\) published the results of a nested case–control study of 150 women with breast cancer; the study examined three ethnic groups—white, black, and Asian. The cases and age-matched controls were drawn from a large cohort of women \((57,040)\) from the San Francisco Bay area, and blood samples were obtained in the late 1960s when DDT was still in use in the United States. Although the mean levels of DDE in the serum of the total study population was similar in cases \((43.3 ± 25.9 \mu g/kg)\) and matched controls \((43.1 ± 23.7 \mu g/kg)\), there were some differences between DDE levels in cases and controls among the different ethnic subgroups. Mean serum levels of DDE were higher in black women with breast cancer \((49.2 ± 25.6 \mu g/kg)\) than in controls \((43.5 ± 21.2 \mu g/kg)\), whereas among Asian women DDE levels were higher in controls \((50.8 ± 27.4 \mu g/kg)\) than in breast cancer cases \((45.1 ± 24.5 \mu g/kg)\). In white women, there was little difference between mean DDE levels in cases \((35.7 ± 23.0 \mu g/kg)\) and controls \((35.0 ± 22.8 \mu g/kg)\), but DDE levels overall were lower in white women compared to black or Asian women regardless of cancer status. These racial/ethnic differences persisted even after adjustment for age and year of examination, BMI, educational level, census block group, working-class composition, poverty level, place of birth, and pregnancy history. When multivariate comparisons were made for the total population, the relative risk expressed as the adjusted OR for breast cancer approached significance only for...
black women (OR 3.85, 95% CI 0.93–16.05, p trend = 0.066; highest total tercile compared with lowest total tercile for serum DDE), not for white or Asian women. However, this association decreased when black terciles were compared (OR 2.16, 95% CI 0.62–7.58, p trend = 0.208; highest black tercile compared with lowest black tercile). Krieger et al. (63) concluded that there was no association between serum DDE levels and breast cancer risk. Others disagree with this conclusion, contending that the elevated OR, though not statistically significant, does support a positive relationship between serum DDE levels and breast cancer risk in black women (107). Krieger and colleagues’ results are controversial. Of the five cohort studies published in late 1990s (64,74–77), none had a sufficiently large number of non-Caucasian women to address whether there were racial/ethnic differences in patterns of breast cancer risk in relation to organochlorine compounds.

Several case–control and correlation studies have attempted to address this issue. In a case–control study of Connecticut women, Zheng et al. (92) reported a significantly higher average (geometric mean) of breast adipose tissue DDE levels for black women (1,926.3 ppb, p < 0.01) than for white women (917.0 ppb). Although a separate multivariate analysis was not conducted to calculate the relative risks in different ethnic subgroups, the OR calculated for the entire study population was adjusted for confounding factors, including race, age, BMI, lactation history, age at menarche, age at first full-term pregnancy, menopausal status, and income. There was no association between levels of breast adipose tissue DDE and breast cancer risk when the highest quartile was compared to the lowest quartile (OR 0.9, 95% CI 0.5–1.5, p trend = 0.46). The authors also noted that the incidence rates for breast cancer in Connecticut have been higher in white than in black women for over 20 years (92). This study does not support an association between higher levels of adipose DDE and breast cancer risk in black women, but it does support previous reports of higher adipose tissue DDE levels in black women than in white women.

The relationship between adipose DDE levels and breast cancer mortality recently has been determined in an ecologic correlation study that included data from 22 U.S. states (108). Data on the DDE concentrations in subcutaneous fat samples were obtained from a U.S. EPA human monitoring report. No information was available on how the fat samples were obtained, stored, or analyzed. Because this was an ecologic study, no information was available on possible confounding factors; the report provided data only on the average DDE levels and sample size by state and race. With the exception of New York State, which had similar adipose DDE levels among blacks and whites, adipose levels of DDE were higher in blacks in all 18 states that reported data on both blacks and whites. The three states with the highest adipose DDE levels in blacks were Arkansas, Texas, and South Dakota. Western and southern states had higher adipose DDE levels than eastern and midwestern states. Mortality from cancer of the breast was inversely correlated with adipose DDE levels both in whites (r = −0.73, p < 0.01) and in blacks (r = −0.50, p < 0.05). None of the other cancer sites studied had positive correlations with adipose tissue DDE levels in whites or blacks, except for liver cancer, which was positively correlated in whites and negatively correlated in blacks. Because the mortality rate for breast cancer is only about 25%, more meaningful data would have included an analysis of breast cancer incidence rates. This study does not support a positive relationship between body burdens of DDE and breast cancer mortality in black or white American women; however, these results must be viewed with caution (because cause-and-effect relationships cannot be inferred from ecologic studies).

If there is a relationship between breast cancer risk and serum organochlorine levels, it could be hypothesized that those populations with the highest body burdens of such chemicals would be priority subpopulations to evaluate. Although most case–control and cohort studies to date do not support an association between serum DDE levels and breast cancer risk in white women, few studies have explored whether women of different racial or ethnic backgrounds may be subpopulations at risk because of higher body burdens of organochlorine compounds. More studies are needed to follow up on the observations of Krieger et al. (63) that black women with breast cancer tend to have higher blood levels of DDE than black women without breast cancer.

**Breast Tumor Characteristics**

Although most of the larger, well-controlled human epidemiologic studies have not supported a relationship between body burden of DDE or DDT and breast cancer risk, others have begun to investigate whether these organochlorines influence the tumor stage, size, or metastatic potential of breast tumors. The age- and lipid-adjusted levels of serum DDE were compared between controls and cases according to stage of tumor in a case–control study by Zheng et al. (84). There were no significant differences between the geometric means for serum DDE in controls (456.2 ppb) and those in cases with breast tumors classified as stage 0-II (455.9 ppb, p < 0.99) or stage III-IV (402.1 ppb, p < 0.57). Hunter et al. (76) did not find an association between breast cancer risk and serum DDE levels in a nested case–control study of 236 breast cancer patients and 236 matched controls from the Nurses’ Health Study cohort (OR 0.72, 95% CI 0.37–1.40, p trend = 0.47). There were also no differences in the levels of DDE in the serum of cases with or without axillary lymph-node involvement [data mentioned but not shown by Hunter et al. (76)]. These results suggest that serum DDE levels are not predictive of the metastatic potential of breast tumors. The results reported by Hunter et al. (76) are in contrast to those in a Canadian case–control study of the aggressiveness of breast tumors in relation to plasma DDE levels reported by Demers et al. (81). Although there were similar mean levels of plasma DDE in the 314 cases (508.9 ± 49.1 µg/kg lipid basis), 218 hospital-based controls (462.7 ± 447.7 µg/kg) and 305 population controls (480.4 ± 408.4 µg/kg), there was evidence of a positive relationship between plasma DDE levels and lymph node involvement in the breast cancer cases. The relative risk for lymph node involvement was significantly elevated in the highest compared to the lowest quintile for plasma p,p’-DDE (OR 2.54, 95% CI 1.20–5.35; adjusted for age, region of residence, BMI, time separating blood sampling from surgery, lactation duration, number of fertile years, and tumor size). Statistical significance was observed only after adjusting for confounding factors, not in the unadjusted data. Tumor size alone was not associated with plasma DDE levels (OR 1.18, 95% CI 0.56–2.21). Whether tissue levels of p,p’-DDE influence the stage of breast cancer, metastatic potential, or lymph node involvement of breast tumors should be studied further, as it is impossible to make a conclusion based on the few studies conducted thus far.

**Polymorphisms**

It is beyond the scope of this review to discuss the numerous studies devoted to identifying polymorphisms that may influence steroid hormone synthesis, degradation, hydroxyla- tion, and the transformation of chemicals into potential carcinogens. Polymorphisms in *CYP17, CYP19, CYP1B1, CYP1A1, COMT,* and *I1307K* have been associated with breast cancer risk (109–115). Whether polymorphisms play a role in breast cancer risk in relation to tissue organochlorines is a topic of emerging interest; few studies have been published to date. Helldsouer et al. (73) found no evidence of a relationship between polymorphisms of *GSTM1, GSTT1, GSTP1, COMT,* and *CYP17,* serum concentrations of DDE and breast cancer risk in a nested
Epidemiologic Studies on Dieldrin and Breast Cancer Risk

The potential of dieldrin to affect breast cancer risk was evaluated by Danish researchers in a prospective nested case–control study that included 7,712 women enrolled in the Copenhagen City Heart Study (74). Serum samples were obtained from participants from 1976 to 1978. In 1996–1997, researchers analyzed serum samples from 240 women who had developed invasive breast cancer and 477 matched breast cancer-free controls for levels of kepone, dieldrin, o,p′-DDT, p,p′-DDT, β-hexachlorocyclohexane, and several PCB congeners. Controls and cases were matched for age, date of examination, and vital status at the examination. Information was obtained on potential confounding factors, including weight, height, number of full-term pregnancies, alcohol consumption, smoking, physical activity, menopausal status, household income, marital status, and education. Independent of breast cancer status, dieldrin was detected in 78% of the women enrolled in the study, with median levels at 24.4 ng/g lipid. There was no relationship of breast cancer risk to serum levels of several PCB congeners, DDE or DDT, or β-hexachlorocyclohexane. The only organochlorine compound associated with a significant increase in breast cancer risk was dieldrin. Women in the highest quartile had double the risk of breast cancer compared to women in the lowest quartile (OR 2.25, 95% CI 1.32–3.84, p trend = 0.003). Relative risks remained unchanged (OR 2.05, 95% CI 1.17–3.57, p trend = 0.01) when adjusted for confounding factors (number of full-term pregnancies and weight). The strengths of this study include its prospective design, that blood samples were obtained before the onset of disease, and its evaluation of a variety of organochlorine compounds.

A subsequent study using the same cohort of Danish women investigated whether breast cancer survival was affected by past exposure to dieldrin (116). To test this hypothesis, researchers obtained serum from 195 women with breast cancer who provided blood samples during two collections, 1976–1978 and 1981–1983, and analyzed the samples for levels of dieldrin. The Causes of Death Registry from the Danish National Board of Health was the source of information for the causes and times of death. Those with the highest blood dieldrin levels from the 1976–1978 blood collection had significantly higher risks of dying than those with the lowest levels [relative risk (RR) 2.78, 95% CI 1.38–5.59, p trend < 0.01; highest quartile compared to lowest quartile]. When the analysis was performed using an average of the blood dieldrin levels from the two collections, the association was even stronger, with a 5-fold higher risk of dying in women from the highest compared to the lowest quartile (RR 5.76, 95% CI 1.86–17.92, p trend < 0.01). In both analyses, relative risks were adjusted for number of positive lymph nodes and tumor size and grade. The authors stated that these findings suggest that past exposure to dieldrin may affect both the risk of developing breast cancer and survival postdiagnosis.

Few studies have evaluated whether serum or adipose dieldrin levels can predict breast cancer risk in American women. Dieldrin was undetectable in adipose tissue samples obtained from five breast cancer cases and five hospital controls without breast cancer enrolled in a study conducted at the Long Island Jewish Medical Center in New Hyde Park, New York (77). A prospective cohort study of women from Missouri failed to find an association between serum dieldrin levels and breast cancer risk (75). Blood samples were donated by 7,224 women in this cohort from 1977 to 1987. During the 9.5-yr follow-up period, 105 women developed breast cancer; each was matched to two controls based on age and date of blood collection. Dieldrin was detected in serum at levels above the limit of detection in 56.2% of the cases and in 61.8% of the controls. The relative risk of breast cancer in relation to serum dieldrin levels was moderately lower when the highest quartile was compared to the lowest quartile (RR 0.6, 95% CI 0.3–1.3, p = 0.38). Because dieldrin was detected only in a little over half the cases in this study, it may not prove to be a useful marker of organochlorine exposure or breast cancer risk in American women. The only organochlorine associated with a higher risk of breast cancer in this study was hexachlorobenzene.

Evidence of the Estrogenicity of Dieldrin

The mechanism by which dieldrin may increase the risk of breast or uterine cancer has not been established, although the ability of dieldrin to act as a xenoestrogen has been given as a possible explanation (79). However, closer examination of the studies that have evaluated the estrogenicity of dieldrin indicate that it is at best a very weak estrogen, and in other tests its ability to act as an estrogen could not be demonstrated.

The most frequently cited evidence for the estrogenicity of dieldrin is the positive response documented in the E-SCREEN assay, which measures cell proliferation in an estrogen-dependent MCF-7 breast tumor cell line (48,118). However, dieldrin elicited an estrogenic response only at the highest concentration tested (10 µM), with no proliferative response in MCF-7 cells at lower doses (118). The relative potency of dieldrin compared to estradiol was calculated to be 1 × 10⁻⁶ from the E-SCREEN assay (118), and 5 × 10⁻⁷ from an ER-mediated luciferase reporter gene
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assay (119). Others have observed minimal estrogenic responses for dieldrin in MCF-7 proliferative assays or in yeast-based ER assays (120). In other experiments conducted by Soto et al. (48), dieldrin was unable to induce progesterone receptor levels but did significantly induce pS2 levels. Researchers have not been able to demonstrate an estrogenic uterotrophic response to dieldrin in in vivo tests using immature rat or mouse uteri (46,121). In ER-binding assays, the ability of dieldrin to displace tritiated estradiol from ER isolated from rodent or rabbit uteri or from alligator ER has been minimal (96,121,122) or tests have yielded negative results (46,123). However, it can be argued that the competitive ER-binding assays may not reflect the capacity of a lipophilic compound with low aqueous solubility to compete for occupancy on ERs isolated from crude uterine preparations. To address this problem a new fluorescence polarization (FP) method that measures the ability of a chemical to displace a high-affinity fluorescent ligand from purified, recombinant human ER was developed by Bolger et al. (96). In the FP assay, dieldrin showed little capacity to inhibit binding of estradiol to purified human ER. Even at the maximal dieldrin concentration tested of 2 × 10−5 µM, estradiol binding was inhibited less than 25%. Although there is little evidence of dieldrin’s estrogenicity, one study has demonstrated that it may act as an antiandrogen. Dieldrin induced a 30% inhibition of tritiated 5α-dehydrotestosterone binding to rat prostate androgen receptor (46).

Potential Routes of Exposure to Dieldrin

One study attempted to document occupational exposure to chemicals in women residing on Cape Cod, Massachusetts. The researchers found that of the 261 breast cancer cases and the 753 controls in the study, only a small number of subjects (2–33) had probable or possible occupational exposure to dieldrin or to a variety of other organochlorines (124). Some estimates have been made of the potential exposure to organochlorines, including dieldrin, in the American diet. The U.S. FDA has published data on the residues of pesticides, including dieldrin, found in foods analyzed as a part of their market-basket surveys conducted from 1991 to 1997 (55). Dieldrin was found in low levels in a variety of foods, including dairy products, meat and poultry products, legumes, eggs, root vegetables such as potatoes, beets, turnips, leafy greens, and vegetables like squash that can come in contact with soil when grown. Maclntosh et al. (58) estimated potential dietary exposures using food consumption data collected as a part of the Nurses’ Health Study and Health Professionals’ Follow-up Study with food residue data from the FDA Total Diet Study. Mean estimated dietary exposures in 1990 for dieldrin were estimated at 0.5 µg/day for males and females, with maximum levels at 4.3 µg/day for females and 16.9 µg/day for males. Estimates of adult intake of dieldrin in the United States or Denmark compiled from data from the early 1980s estimated the average daily intake of dieldrin at 0.02 µg/kg body weight (60 kg female = 1.2 µg/day dieldrin; 80 kg male = 1.6 µg/day dieldrin) (125). This is generally consistent with the observation that dieldrin levels have been declining in food samples and human tissues since its ban for agricultural use in the mid-1970s and its ban for termiteicide use in the late 1980s (62).

Because of its persistence in soil and low mobility, dieldrin is seldom detected as a water contaminant in the United States. A study conducted by the U.S. Geological Survey from 1993 to 1995 in 20 major hydrologic basins throughout the United States reported very low frequencies of dieldrin in groundwater, regardless of type of land use (126). Overall, only 1.4% of the 1,034 sites sampled had detectable levels of dieldrin. The type of land use with the highest percent detection was orchards and vineyards, with an average of 3.3% of the sites with detectable levels of dieldrin. Dieldrin has been detected in areas with past historical use. In a study of 50 shallow wells from Suffolk County, New York, which has a sandy and gravel aquifer susceptible to contamination, New York State Class GA standards for dieldrin in groundwater were exceeded in eight wells (standard is 0.004 µg/L for dieldrin). The highest dieldrin level detected in a Suffolk County well was 0.1 µg/L (127).

Conclusions and Needs for Further Research

Most of the nested case-control and case-control studies conducted since 1996 have failed to confirm earlier observations of a significant positive relationship between serum or adipose tissue levels of DDE or DDT and breast cancer risk. Most of these studies have been conducted using Caucasian women from the United States, Canada, or Europe. Because some studies have documented a tendency for black women in the United States to have higher serum or adipose DDE levels than white women, further studies are needed to determine whether black women have a higher risk for breast cancer associated with body burdens of DDE.

One reason for the lack of association between blood or tissue DDE levels and breast cancer risk may be the route of exposure to different forms of DDT and its metabolites of varying estrogenicity. Specifically, in Western populations, the primary contribution to body levels of DDE may be via p,p′-DDE ingested preformed from the diet rather than exposure to technical DDT, which contains the more estrogenic congener o,p′-DDT.

Although it does not appear that DDE predicts breast cancer risk in white Western women in North America or in Europe, there are other populations with more recent exposure to DDT that it may be prudent to evaluate. Some of the studies conducted in countries with more recent DDT use have not found a relationship to breast cancer risk (79,80,83), but two studies, conducted in Colombia, South America (82), and in Mexico City (96), have found an elevated risk of breast cancer in women with higher serum levels of DDE. To test whether the estrogenic o,p′-DDT found in technical DDT is capable of supporting the growth of estrogen-dependent breast tumors, further studies should be conducted in Third World countries, such as India, that have a long and continuing history of DDT use.

It is not possible to conclude whether serum dieldrin levels are associated with breast cancer risk until the results of other studies become available. Studies done to date have found a relationship between serum dieldrin levels and breast cancer risk in Danish but not American women. An area of research that should be pursued is whether body burdens of DDE or dieldrin are associated with tumor size, metastatic potential, or morbidity from breast cancer. Other emerging areas of research will be the relationship to polymorphisms and organochlorine levels to breast cancer risk.

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