Environmental factors and breast cancer

S H Safe and A McDougal
Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas 77843-4466, USA

Introduction

Breast cancer is the leading cancer among women in the USA and most Western countries, and the incidence rates are approximately 100 per 100 000 population. From 1970 to 1990 there has been a 117 and 50% increase in breast cancer incidence and mortality respectively in the USA; however, the age-adjusted increased incidence and mortality are 21 and 3% respectively within this same time-period. These increases have primarily been observed in older postmenopausal women (Sondik 1994, Ries 1995). There are also major geographical differences in breast cancer incidence between Western (high incidence) and Asian (low incidence) countries. However, several studies have demonstrated that breast cancer incidence increases dramatically in Asian immigrants in the USA, suggesting that differences between these populations are not due to race but to other ‘environmental factors’ including diet.

An important component in the contribution of ‘environmental factors’ appears to be a woman’s overall lifetime exposure to estrogens (Pike et al. 1993, Hulka et al. 1994). The following hormonally related factors increase the risk for breast cancer: late age at menopause, null parity, late age at first birth, early age at menarche and estrogen replacement therapy. Some of these factors involving lifetime estrogen exposure may also be responsible, in part, for differences in breast cancer incidence between China and the USA; for example, the average age of menarche is 17 and 12.8 years in the two countries respectively. It has been difficult to assess the role of diet in breast cancer; for example, the effects of dietary fat intake are equivocal; however, body mass index (obesity) is a risk factor for women over 31. The role of fat and other dietary components in breast cancer is complex and requires further research (Marshall 1993, Willett & Hunter 1994). It has also been suggested that mutagenic aromatic amines such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine which are formed in grilling or frying fish or meat may constitute a group of dietary carcinogens which induce mammary carcinogenesis (Ghoshal et al. 1994, Nagao et al. 1994). At present, there are insufficient epidemiological data to link exposure to cooking-derived carcinogens with breast cancer and this is an area of research which could further define hormone-independent risk factors. It has recently been hypothesized that industrial-derived estrogenic compounds (xenoestrogens) may also be a risk factor for breast cancer in women (Davis et al. 1993, Davis & Bradlow 1995) and the validity of this hypothesis will be discussed in this paper.

Organochlorine xenoestrogens and their role in breast cancer

Davis et al. (1993) have hypothesized that human exposure to xenoestrogens may be a preventable cause of breast cancer. In recent years, several studies have identified structurally diverse industrial chemicals which bind to the estrogen receptor (ER) and/or induce estrogenic responses in several estrogen-responsive assay systems (Welch et al. 1969, Bitman & Cecil 1970, Hammond et al. 1979, Berthois et al. 1986, Korach et al. 1988, Soto et al. 1991, 1994, 1995, Krishnan et al. 1993, White et al. 1993, Jobling et al. 1995). Some of these compounds include organochlorine pesticides such as o,p'-DDT, p,p'-DDE, kepone, toxaphene, endosulfan, dieldrin, polychlorinated biphenyl (PCB) mixtures and congeners, hydroxy-PCBs, bisphenol-A, nonylphenol and some
phthalates (Fig. 1). Two case-control studies provided important initial data which supported the xenoestrogen hypothesis: namely, PCB levels in breast tumor tissue or serum levels of \( p,p' \)-DDE were elevated in breast cancer patients versus controls (Falck et al. 1992, Wolff et al. 1993). In contrast, a subsequent larger study with 150 patients and 150 controls reported that neither serum PCB nor \( p,p' \)-DDE levels were increased in women with breast cancer (Krieger et al. 1994). The results of several studies are summarized in Table 1 and meta-analysis of these data indicates that DDE or PCB levels were not significantly elevated in breast cancer patients (Key & Reeves 1994, Adami et al. 1995, Ahlborg et al. 1995). A recent nested case-control study among women exposed to polybrominated biphenyls (PBBs) in Michigan reported that serum PBB levels were higher in the breast cancer cases compared with controls (Henderson et al. 1995). For example, women with 2.0 to 3.0 and >4.0 ppb serum PBB levels had an increased risk for breast cancer (odds ratios of 3.5 and 3.1 respectively) compared with women with lower serum PBB levels (≤2.0 ppb). Serum levels of DDE or PCBs in these patients were not reported. The rationale and significance of elevated organochlorine levels in some groups of breast cancer patients are unclear; it has been suggested that since concentrations of these compounds are higher in fatty foods and fish, elevated levels of these compounds in some studies may be indicative of a localized dietary risk factor (Safe 1995b). The results of several ongoing studies among native populations and residents of Long Island (New York) may provide important new information regarding levels of various contaminants in breast cancer patients.

Davis, Bradlow and coworkers have also proposed that estrogenic organochlorine pesticides and other xenoestrogens may affect breast cancer by decreasing 17β-estradiol 2-hydroxylase and increasing \( E_2 \) 16α-hydroxylase activities resulting in elevated 16α-hydroxyestrone (HO-E\(_1\))/2-HO-E\(_1\) metabolite ratios (Bradlow et al. 1991, 1995, Davis et al. 1993, Davis & Bradlow 1995). Previous studies have reported that 16α-hydroxy-\( E_2 \) is a potent estrogen whereas 2-hydroxy-\( E_2 \) exhibited partial ER antagonist activities (Schneider et al. 1984, Swaneck & Fishman 1988). Recent studies using MCF-7 human breast cancer cells reported that several putative estrogenic pesticides, including endosulfan, \( γ \)-benzene hexachloride, kepone, \( p,p' \)-DDE, \( o,p' \)-DDE, \( o,p' \)-DDT, atrazine, 2,2',4,4',5-pentachlorobiphenyl, and the mammary carcinogen 7,12-dimethylbenz(a)anthracene (DMBA), all decreased \( E_2 \) 2-hydroxylase and increased \( E_2 \) 16α-hydroxylase activities and the 16α-HO-E\(_1\)/2-HO-E\(_1\) metabolite ratios (Bradlow et al. 1995). In contrast, indole-3-carbinol (I3C), a compound in cruciferous vegetables which inhibits development and/or growth of mammary tumors in both in vivo and in vitro studies.

![Figure 1](image-url)  
**Figure 1** Structures of organohalogen endocrine disruptors.
Table 1 Organohalogen levels and breast cancer: case-control studies.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cases/controls</th>
<th>Levels (ppm) (cases/controls)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDE/PCB (biopsies)</td>
<td>14/21</td>
<td>1.23/1.25 (DDE) 3.89/3.93 (PCB)</td>
<td>Unger et al. 1984</td>
</tr>
<tr>
<td>PCB (dead cases)</td>
<td>18/33</td>
<td>6.47/5.12</td>
<td></td>
</tr>
<tr>
<td>DDE (mammary tissue)</td>
<td>41/33</td>
<td>0.96/0.98</td>
<td>Mussalo-Rauhamaa et al. 1990</td>
</tr>
<tr>
<td>PCB (mammary tissue)</td>
<td>41/43</td>
<td>1.05/1.30</td>
<td></td>
</tr>
<tr>
<td>DDE (mammary tissue)</td>
<td>20/20</td>
<td>1.877/1.179</td>
<td>Falck et al. 1992</td>
</tr>
<tr>
<td>PCB (mammary tissue)</td>
<td>20/20</td>
<td>1.669/1.105</td>
<td></td>
</tr>
<tr>
<td>DDE (serum)</td>
<td>58/171</td>
<td>11.0/7.7 (ng/ml)</td>
<td>Wolff et al. 1993</td>
</tr>
<tr>
<td>PCB (serum)</td>
<td>58/171</td>
<td>8.0/6.7 (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>DDE (mammary tissue)</td>
<td>18/17</td>
<td>1.371/0.765</td>
<td>Dewailly et al. 1994</td>
</tr>
<tr>
<td>PCB (mammary tissue)</td>
<td>18/17</td>
<td>0.368/0.397</td>
<td></td>
</tr>
<tr>
<td>DDE (serum)</td>
<td>150/150</td>
<td>0.043/0.043</td>
<td>Krieger et al. 1994</td>
</tr>
<tr>
<td>PCB (serum)</td>
<td>150/150</td>
<td>0.0044/0.0048</td>
<td></td>
</tr>
<tr>
<td>PBB (serum)</td>
<td>20/190</td>
<td>0.003/0.002 (median values)</td>
<td>Henderson et al. 1995</td>
</tr>
</tbody>
</table>

Figure 2 Induction of E\(_2\) 2-hydroxylase activity by I3C and ICI 164,384 in MCF-7 human breast cancer cells. Cells were incubated for 2 or 48 h with 10 µM I3C or ICI 164,384 and 2-[\(^3\)H]E\(_2\) and activity was determined by radiometric assay of tritiated water.
induced E₂ 2-hydroxylase and decreased E₂ 16α-hydroxylase activities and the 16α-HO-E₂/2-HO-E₁ metabolite ratios in MCF-7 cells. Based on these results, it was suggested that ‘the ratio of 16α-OH-E₂/2-OH-E₁ may provide a marker for risk of breast cancer’ (Bradlow et al. 1995). The utility of this MCF-7 cell assay was re-investigated in this laboratory using 2-[³H]E₂ as a substrate for the radiometric determination of E₂ 2-hydroxylase activity (by counting ³H₂O). The E₂ 2-hydroxylase activities previously reported after treatment of MCF-7 cells with various pesticides, DMBA and I3C for 48 h were also noted in this laboratory; however, with few exceptions, these same responses were observed within 2 h after treatment with the various chemicals. These results suggest that the altered E₂ 2-hydroxylase activities were not due to P450 induction but to direct interactions of the chemical substrates with the P450 system (Fig. 2). Subsequent studies have shown that benzo[a]pyrene (BaP), a mammary carcinogen, induced E₂ 2-hydroxylase activity in MCF-7 cells and this is consistent with results of previous studies which have reported that BaP induced CYP1A1-dependent activity in MCF-7 cells (Chaloupka et al. 1992). In addition, we have shown that the antiestrogen ICI 164,384 (Wakeling 1995) also decreased E₂ 2-hydroxylase activity (Fig. 2). These results indicate that modulation of E₂ 2-hydroxylase activities is not a predictive assay for putative estrogenic pesticides or mammary carcinogens and the linkage between the activity of various chemicals in this MCF-7 cell assay and mammary carcinogenesis is questionable.

Several other studies have been cited as support for the hypothesis that some organochlorine pesticides and related xenoestrogens may play a role in development of breast cancer. For example, atrazine, a widely used herbicide was listed as a ‘proved xenoestrogen’ (Davis & Bradlow 1995). In female Sprague-Dawley rats, this herbicide decreased the latency period for development of age-dependent mammary tumors; however, this response was not observed in female Fisher rats and it was suggested that atrazine and related compounds may influence estrus and luteinizing hormone regulation via non-estrogenic pathways (Wetzel et al. 1994). Moreover, a recent study in this laboratory using several diagnostic estrogen-responsive assays concluded that atrazine and simazine were not estrogenic (Connor et al. 1996) and this was also confirmed in the E-screen assay (Soto et al. 1995). Davis et al. (1993) also cited a study by Scribner & Mottet (1981) which showed that DDT increased the incidence of mammary tumors in male mice treated with acetamidophenanthrene. However, an earlier study by Silinskas & Okey (1975) reported that DDT decreased the incidence of DMBA-induced mammary tumors in female rats. Thus, the laboratory animal evidence which supports the role of DDT and related compounds as mammary carcinogens is equivocal since many organochlorine compounds both enhance and inhibit carcinogenesis in animal models.

The correlation between environmental exposure to xenoestrogens and other chemical contaminants and cancer is difficult to prove from epidemiological studies since the major route of exposure, the human diet, results in the intake of complex mixtures of xenobiotics and natural compounds. The linkage between exposure to specific industrial chemicals such as DDT or PCBs and development of cancer is more readily observed in more highly exposed groups of workers. Epidemiological studies on women highly exposed to PCBs or DDT do not show an increased incidence of mammary cancer (reviewed by Ahlborg et al. 1995) suggesting that low level environmental exposures to these chemicals are probably not etiologic agents for breast cancer.

**Organochlorine compounds as antiestrogens**

The hypothesis that xenoestrogens may be a preventable cause of breast cancer (Davis et al. 1993) was based, in part, on two observations; namely, several organochlorine pesticides were identified as estrogens and DDE or PCB levels were elevated in some groups of breast cancer patients (Falck et al. 1992, Wolff et al. 1993). It was subsequently pointed out that other organochlorine pollutants typified by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) had been extensively characterized as antiestrogens and these compounds may possibly counteract some estrogen-induced responses (Safe 1994). Kociba et al. (1978) first reported that age-dependent spontaneous mammary and uterine tumor formation in female Sprague-Dawley rats was decreased in animals administered TCDD (0.001-0.1 μg/kg per day) in the diet for 2 years. Subsequent studies in

The antiestrogenic activities of TCDD resembled those reported for ‘pure’ antiestrogens such as ICI 164,384 which act through the ER. Both TCDD and ICI 164,384 inhibit a broad spectrum of E2-induced responses and downregulate ER protein levels in MCF-7 human breast cancer cells. In contrast, the

![Diagram of possible mechanisms of antiestrogenic effects of TCDD](image)

**Figure 3** Possible mechanisms of the antiestrogenic effects of TCDD (Safe 1995a). AhR directly binds DREs in the 5′-flanking regions of ER-induced genes (pathway 1); TCDD induces a factor F that (a) degrades the nuclear ER (pathway 2), (b) inhibits estrogen-induced mitogen activity (pathway 3), and (c) exhibits direct antimitogenic activity (pathway 4). Arnt, Ah receptor nuclear translocator. This diagram was presented in the PhD thesis of M Moore, Texas A&M University.
latter compound competitively binds to the ER and is an ER antagonist (Wakeling 1995). TCDD does not bind to the ER, PR or other steroid hormone receptors, and mechanistic studies indicate that TCDD and related compounds elicit diverse biochemical and toxic responses through initial binding to the aryl hydrocarbon receptor (AhR) (Safe 1995a). The AhR binds diverse structural classes of HAHS and polynuclear aromatic hydrocarbons (PAHS) and responses are induced through a signaling pathway similar to that observed for other ligand-induced transcription factors (Swanson & Bradfield 1993, Whitlock 1993, Safe 1995a). After ligand binding, the nuclear AhR forms a unique heterodimeric complex with the AhR nuclear translocator protein and interaction of the heterodimer with cis genomic dioxin or xenobiotic responsive elements (DREs or XREs) located in 5'-promoter regions of target genes results in transactivation. The role of the AhR in mediating the antiestrogenic activities of various structural classes of agonists has been confirmed in several studies (Safe 1995a). For example, structure-activity studies with several AhR agonists have shown a correlation between structure-AhR binding versus structure-antiestrogenicity relationships; expression of a functional nuclear AhR complex is required for ligand-induced antiestrogenicity. It should also be noted that AhR agonists also inhibit growth factor-induced proliferation of human breast cancer cells (Fernandez & Safe 1992, Liu et al. 1992), and Fig. 3 illustrates possible pathways for inhibition of E2-induced responses via the AhR. Interaction with an inhibitory DRE (iDRE) identified in the 5'-promoter region of the cathepsin D gene has previously been reported and this pathway may be involved for several other genes including pS2 (Zacharewski et al. 1994). Thus, AhR agonists are an important class of antiestrogens and included among

Table 2 Mass balance dietary exposure to different classes of estrogenic and antiestrogenic chemicals.

<table>
<thead>
<tr>
<th>Chemical (source)</th>
<th>Mass (ng/day)</th>
<th>Estrogen or antiestrogen(^a) equivalents (ng/day) (rel. pot. factor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioflavonoids</td>
<td>100 000 000(^b)</td>
<td>10 000-102 000</td>
</tr>
<tr>
<td>(fruits, vegetables, nuts)</td>
<td>1 020 000 000</td>
<td>(10(^{-4}))</td>
</tr>
<tr>
<td>Organochlorine pesticides</td>
<td>2500(^c)</td>
<td>&lt;0.25 (10(^{-4}))</td>
</tr>
<tr>
<td>Antiestrogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCDD and related compounds (including PCBs)</td>
<td>0.1-0.3(^d)</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>PAHs in cooked foods</td>
<td>1200-5000(^e)</td>
<td>1.2-5.0 (10(^{-4}))</td>
</tr>
<tr>
<td>I3C (in cruciferous vegetables - 25 g Brussels sprouts)</td>
<td>ca. 400 000 to 1 600 000(^f)</td>
<td>40-160 (10(^{-4}))</td>
</tr>
</tbody>
</table>

\(^a\)The estrogen/antiestrogen equivalents data for bioflavonoids and organochlorine pesticides utilized relative potency factors (rel. pot. factor) reported by Soto et al. (1994, 1995). The relative antiestrogenic potency factors for TCDD, I3C and PAHs were also derived from studies in breast cancer cells (Chaloupka et al. 1992, Liu et al. 1994, Safe 1995a, b). \(^b\)In vitro studies with TCDD and E2 indicate that these compounds are as equipotent as antiestrogens and estrogens respectively for most responses and therefore one antiestrogen equivalent (using TCDD equivalents) is similar to one estrogen equivalent (using E2 equivalents) (Safe 1995a). \(^c\)Verdeal & Ryan 1979. \(^d\)Winter 1992. \(^e\)US Environmental Protection Agency 1994. \(^f\)Vaessen et al. 1988. \(^g\)Bjeldanes et al. 1991 (average intake of 2.5-12.5 \(\mu\)mol I3C/day).
this group of chemicals are not only organochlorine pollutants but also natural compounds such as I3C and related hetero-PAHS present in cruciferous vegetables and PAHs which are formed in cooking fish and meat.

**Comparative human dietary exposures to estrogenic and antiestrogenic chemicals**

Hazard and risk assessment of the potential dietary impact of xenoestrogens on breast cancer incidence must also take into account exposures to other estrogenic and antiestrogenic compounds in the diet. The overall dietary exposure to weakly estrogenic organochlorine compounds and other xenoestrogens has not been determined. However, dietary intakes of estrogenic organochlorine pesticides are routinely estimated (Winter 1992) in a market-basket survey which analyzes pesticide levels in various food products (Table 2). Based on recent data, the estimated daily intake of DDT/DDE (primarily p,p'-DDE), toxaphene and dieldrin is 2.5 μg/day. Average daily intakes of other xenoestrogens are unknown. There is evidence for human exposures to bisphenol-A, phthalates and nonylphenol (Mayer et al. 1972, Marcomini et al. 1988, Olea et al. 1996) but average daily intakes of these compounds are unknown, although relatively high exposure groups for bisphenol A and phthalates have been identified (Thomas & Thomas 1984, Olea et al. 1996). Bioflavonoids are a major dietary source of natural estrogenic compounds which have been identified in fruits, nuts and vegetables, and estimated human intakes of estrogenic bioflavonoids range from 100 to 1000 mg/day (Kuhnau 1976, Verdeal & Ryan 1979). Estrogenic lignans have also been detected in humans (Aldercreutz et al. 1993), and other estrogenic compounds including E2 are present in various food products; however, average daily intakes are unknown.

The human diet also contains a diverse spectrum of chemicals which are known to protect against breast cancer in animal models and these include various antioxidant terpenoids such as limonene (Ames et al. 1995). As noted in the previous section, TCDD and related HAHs also exhibit antiestrogenic activity and dietary levels of HAHs and their corresponding TCDD or toxic (or antiestrogen) equivalents have been determined (see Table 2) (US Environmental Protection Agency 1994). Other AhR agonists in food include PAHs and I3C, and average dietary levels of these compounds have also been determined (Vaessen et al. 1988, Bjeldanes et al. 1991, Menzie et al. 1992). Dietary intakes of various classes of estrogens and antiestrogens are summarized in Table 2 and the results demonstrate that, based on mass intakes, the weakly estrogenic organochlorine pesticides constitute only a minor fraction of the daily intake of estrogenic compounds. It should be noted that several groups of women with relatively high intakes of potent estrogenic drugs (e.g. hormonal contraceptives and hormone replacement therapy) appear to be at minimal risk for breast cancer (Hulka et al. 1994). Based on the low human dietary exposures to weakly estrogenic organochlorine pesticides, it seems highly unlikely that these compounds increase the risk for breast cancer.

**Acknowledgements**

The financial assistance of the National Institutes of Health (ES04176) and the Texas Agricultural Experiment Station is gratefully acknowledged. S S is a Sid Kyle Professor of Toxicology at Texas A&M University.

**References**


Indolo[3,2-b]carbazole; a dietary factor which exhibits both antiestrogenic and estrogenic activity. *Journal of the National Cancer Institute* **86** 1758-1765.


Ries LAG 1995 Stat bite: top 5 cancers for females and males in the US. *Journal of the National Cancer Institute* **87** 867.


---


Safe S 1995b Environmental and dietary estrogens and human health - is there a problem? *Environmental Health Perspectives* 103 346-351.


Silinskas KC & Okey AB 1975 Protection by 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) against mammary tumors and leukemia during prolonged feeding of 7,12-dimethylbenz(a)anthracene to female rats. *Journal of the National Cancer Institute* 55 653-657.


Soto AM, Chung KL & Sonnenschein C 1994 The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. *Environmental Health Perspectives* 102 380-383.


Tiwari RK, Guo L, Bradlow HL, Telang NT & Osborne MP 1994 Selective responsiveness of breast cancer cells to indole-3-carbinol, a chemopreventative agent. *Journal of the National Cancer Institute* 86 126-131.


Reviews in Environmental Contamination and Toxicology 127 23-67.

Wolff MS, Toniolo PG, Leel EW, Rivera M & Dubin N 