Environmental and Dietary Estrogens and Human Health: Is There a Problem?

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It has been hypothesized that organochlorine pesticides and other environmental and dietary estrogens may be associated with the increased incidence of breast cancer in women and decreased sperm concentrations and reproductive problems in men. However, elevation of organochlorine compounds such as dichlorodipehenyldichloroethylene (DDE) and polychlorinated biphenyls (PCBs) in breast cancer patients is not consistently observed. Reanalysis of the data showing that male sperm counts decreased by over 40% during 1940 to 1990 indicated that inadequate statistical methods were used and that the data did not support a significant decline in sperm count. Humans are exposed to both natural and industrial chemicals which exhibit estrogenic and antiestrogenic activities. For example, bioflavonoids, which are widely distributed in foods, and several industrial compounds, including organochlorine pesticides and various phenolic chemicals, exhibit estrogenic activity. Humans are also exposed to chemicals which inhibit estrogen-induced responses such as the aryl hydrocarbon receptor (AhR) agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related chlorinated aromatics, polynuclear aromatic hydrocarbon combustion products, and indole-3-carbinol, which is found in cruciferous vegetables. Many of the weak estrogenic compounds, including bioflavonoids, are also antiestrogenic at some concentrations. A mass balance of dietary levels of industrial and natural estrogens, coupled with their estimated estrogenic potencies, indicates that the dietary contribution of estrogenic industrial compounds is 0.0000025% of the daily intake of estrogenic flavonoids in the diet. Moreover, dietary levels of antiestrogen equivalents (industrial or natural) are significantly higher than the estrogen equivalents of organochlorine pesticides. The suggestion that industrial estrogenic chemicals contribute to an increased incidence of breast cancer in women and male reproductive problems is not plausible. *Key words*: antiestrogens, dietary estrogens, estrogen equivalents, pesticides, polychlorinated biphenyls, TCDD. *Environ Health Perspect* 103: 346–351 (1995)

Recent reports have suggested that background levels of industrial chemicals and other environmental pollutants may play a role in development of breast cancer in women and decreased male reproductive success as well as the reproductive failures of some wildlife species (*1–6*). These suggestions have been supported by articles in the popular and scientific press (*7–13*) and by a television documentary (*14*) which have described the perils of exposure to endocrine-disrupting chemicals such as estrogenic organochlorine pesticides and pollutants. During the past two decades, environmental regulations regarding the manufacture, use, and disposal of chemicals have resulted in significantly reduced emissions of most industrial compounds and their by-products. Levels of the more environmentally stable organochlorine pesticides and pollutants are decreasing in most ecosystems including the industrialized areas around the Great Lakes in North America (*15–18*). Decreased levels of organochlorine compounds correlates with the improved reproductive success of highly susceptible fish-eating water birds in the Great Lakes region (*19*). This article reviews key papers that have been used to support the hypotheses that environmental estrogens play a role in the increased incidence of breast cancer in women and decreased sperm counts in males. Environmental/dietary estrogens and antiestrogens are identified and intakes of "estrogen equivalents" are estimated to compare the relative dietary impacts of various classes of estrogenic chemicals.

Role of Estrogens in Breast Cancer and Male Reproductive Problems

Concerns regarding the role of environmental and dietary estrogens as possible contributors to the increased incidence of breast cancer were fueled by several reports that showed elevated levels of organochlorine compounds in breast cancer patients (*20–24*). The results presented in Table 1 summarize some of these studies that compare levels of organochlorine compounds in breast tissue or serum from breast cancer patients and controls. Polychlorinated biphenyls (PCBs) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) are the two most abundant organochlorine pollutants identified in all human tissues with high frequencies. In one Scandinavian study, levels of DDE or PCBs in adipose tissue from breast samples were not significantly different in breast cancer patients compared to controls (*20*). In another study in Finland, β-hexachlorocyclohexane levels were elevated in breast cancer patients (*21*); however, this compound was not detected in adipose tissue of some individuals in the patient and control groups and has a relatively low frequency of detection in human tissue samples. Falck and co-workers reported that PCB levels were elevated in mammary adipose tissue samples from breast cancer patients in Connecticut (*22*). In contrast, serum levels of DDE (but not PCBs) were significantly elevated in breast cancer patients enrolled in the New York University Women's Health Study (*23*). DDE (but not PCB) levels were also elevated in estrogen receptor (ER)-positive but not ER-negative breast cancer patients from Quebec compared to levels in women with benign breast disease (*24*). It was initially concluded by Wolff and co-workers that "these findings suggest that environmental chemical contamination with organochlorine residues may be an important etiologic factor in breast cancer" (*22*). The correlations reported in the two U.S. studies (*22,23*) heightened public and scientific concern regarding the potential role of these compounds in development of breast cancer. These observations undoubtedly reinforced advocacy by some groups for a ban on the use of all chlorine-containing chemicals. However, the proposed linkage between PCBs and/or DDE and breast cancer is questionable for the following reasons:

- Most studies with PCBs indicate that these mixtures are not estrogenic, and the weak estrogenic activity observed for lower chlorinated PCB mixtures may be due to their derived hydroxylated metabolites;
- *p,p′*-DDE, the dominant persistent metabolite of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p′*-DDT), is not estrogenic, and levels of *o,p*'-DDT, the estrogenic member of the DDT family, are low to nondetectable in most environmental samples;

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Table 1. Organochlorine levels in breast cancer patients

Country	Organochlorine compound	Patient group (*n*)	Levels	Reference
Finland	β-Hexachlorocyclohexane levels elevated in breast cancer patients (breast tissue)	Breast cancer patients (24) Controls (16)	0.13 ± 0.06 ppm 0.08 ± 0.03 ppm	(*21*)
Norway	DDT and PCB levels comparable in patients and controls (breast tissue)	Breast cancer patients (18) Controls (35)	6.47 ± 2.35 ppm PCB 5.12 ± 2.38 ppm PCB; 1.97 ± 2.24 ppm DDT	(*20*)
USA (Connecticut)	PCB levels elevated in breast cancer patients (breast tissue)	Breast cancer patients (20) Controls (20)	1669 ± 894 ppm PCB 1105 ± 424 ppb PCB	(*23*)
USA (New York)	DDE levels elevated in breast cancer patients (serum)	Breast cancer patients (58) Controls (171)	11.0 ± 9.1 ng/mL 7.7 ± 6.8 ng/mL	(*22*)
USA (California)	PCB and DDE levels comparable in patients and controls (serum)	Breast cancer patients (150) Controls (150)	4.4 ± 1.8 ppb PCB; 43.3 ± 25.9 ppb DDE 4.8 ± 2.5 ppb PCB; 43.1 ± 23.7 ppb DDE	(*27*)
Canada (Quebec)	DDE levels increased in estrogen receptor-positive patients (breast tissue)	Breast cancer patients (9) Controls (17)	2132 ± 2050 ppm 765 ± 527 ppm	(*24*) (*24*)

- Epidemiology studies of individuals occupationally exposed to relatively high levels of DDT (*25*) or PCBs (*26*) do not show a higher incidence of breast cancer; and
- No single class of organochlorine compounds was elevated in all studies, suggesting that other factors may be critical for development of breast cancer.

Krieger and co-workers (*27*) recently reported results from a nested case–control study of women from the San Francisco area which showed that there were no differences in serum DDE or PCB levels between breast cancer patients and control subjects. The authors concluded that "the data do not support the hypothesis that exposure to DDE and PCBs increases risk of breast cancer" (*27*: p. 589). This was duly noted in *Time* magazine (*28*) by a three-line statement in "The Good News" section. Moreover, combined analysis of the 6 studies which report PCB and DDE levels in 301 breast cancer patients and 412 control patients showed that there were no significant increases in either DDE or PCB levels in breast cancer patients versus controls (*29*).

The second major link between environmental/dietary estrogens and human disease was precipitated by an article published in the *Lancet*, in which Sharpe and Skakkebaek (*5*) hypothesized that increased estrogen exposure may be responsible for falling sperm counts and disorders of the male reproductive tract. Unlike the proposed link between environmental estrogens and breast cancer, this hypothesis was not based on experimentally derived measurements of increased levels of any estrogenic compounds in males. Previous studies with diethylstilbestrol, a highly potent estrogenic drug, showed that *in utero* exposure results in adverse effects in male offspring (*30*), and the authors' hypothesized that *in utero* exposure to environmental/dietary estrogens may also result in adverse effects in male offspring. A critical experimental component supporting the authors' hypothesis was their analysis of data from several studies which indicated that male sperm counts had decreased by over 40% during the past 50 years (*31*). These observations, coupled with the hypothesis that environmental estrogens including organochlorine chemicals were possible etiologic agents, were reported with alarm in the popular and scientific press (*7–12*) and in a BBC television program entitled "Assault on the Male: a Horizon Special" (*14*). Subsequent and prior scientific studies have cast serious doubts on both the hypothesis (*5*) and the observed decrease in male sperm counts (*31*). In 1979, Macleod and Wang (*32*) reported that there had been no decline in sperm counts, and reanalysis of the data presented by Carlsen and co-workers showed that sperm counts had not decreased from 1960 to 1990 (*33*). Thus, during the time in which environmental levels of organochlorine compounds were maximal, there was not a corresponding decrease in sperm counts. Moreover, a reevaluation of the sperm concentration data was recently reported by Brownwich et al. (*34*) in the *British Medical Journal*, and their analysis suggested that the decline in sperm values in males was a function of the choice of the normal or reference value for sperm concentrations. The authors contend that their analysis of the data does "not support the hypothesis that the sperm count declined significantly between 1940 and 1990" (*34*: p. 19).

These results suggest that the increasing incidence of human breast cancer is not related to organochlorine environmental contaminants and that decreases in sperm counts is highly debatable. Nevertheless, human populations are continually exposed to a wide variety of environmental and dietary estrogens, and these compounds clearly fit into the category of "endocrine disrupters." The remainder of this article briefly describes the different structural classes of both environmental and dietary estrogens and quantitates human exposures to these compounds.

Synthetic Industrial Chemicals with Estrogenic Activity

The estrogenic activities of different structural classes of industrial chemicals were reported by several research groups in the late 1960s and 1970s in which *o,p′*-DDT and other diphenylmethane analogs (Fig. 1) and the insecticide kepone were characterized as estrogens (*35–38*). Subsequent studies have confirmed the estrogenic activity of *o,p*-DDT and related compounds (*39*) whereas the *p,p′*-substituted analogs were relatively inactive (*36,37*). In addition, *p,p′*-methoxychlor and its hydroxylated metabolites elicit estrogenic responses (*39,40*). Ecobichon and Comeau (*41*) investigated the estrogenic activities of commercial PCB mixtures (Aroclors) and individual congeners in the female rat uterus and reported estrogenic responses for some Aroclors and individual congeners. Studies in this laboratory showed that a number of commercial PCBs did not significantly increase secretion of procathepsin D, an estrogen-regulated gene product, in MCF-7 human breast cancer cells (*42*). It should be noted that several hydroxylated PCBs bind to the ER, and it is possible that *para*-hydroxylated PCB metabolites may be the active estrogenic compounds associated with lower chlorinated PCBs (*43*). A recent study reported that several additional organochlorine pesticides including endosulfan, toxaphene, and dieldrin exhibit estrogenlike activity and induce proliferation of MCF-7 human breast cancer cells (*44*).

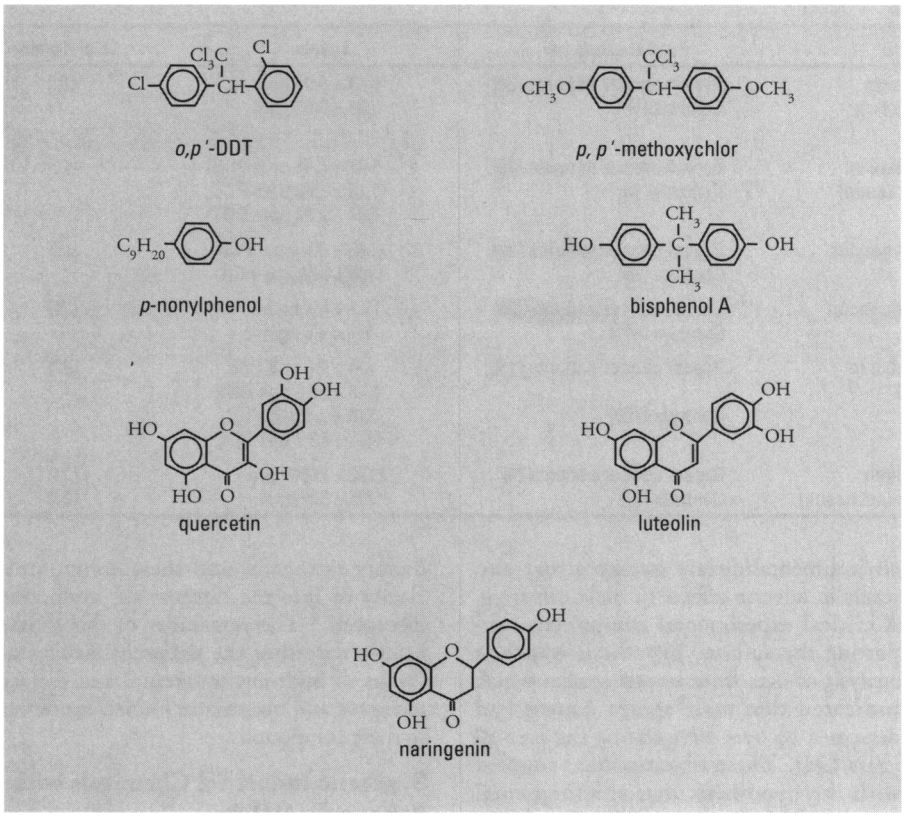

Figure 1. Structures of environmental estrogens (*o,p'*-DDT, *p,p'*-methoxychlor, *p*-nonylphenol, and bisphenol A) and estrogenic bioflavonoids (quercetin, naringenin, and luteolin).

Other industrial chemicals or intermediates that have been identified as estrogenic compounds include bisphenol-A (Fig. 1), a chemical used in the manufacture of polycarbonate-derived products (*45*); phenol red, a pH indicator used in cell culture media (*46*); and alkyl phenols and their derivatives, which are extensively used for preparation of polyethoxylates in detergents (*47,48*).

Natural Estrogenic Compounds

Human exposure to estrogenic chemicals is not confined to xenoestrogens derived from industrial compounds. Several different structural classes of naturally occurring estrogens have been identified, including plant bioflavonoids (Fig. 1) and various mycotoxins including zearalenone and related compounds (*49–52*). The plant bioflavonoids include different structural classes of compounds which contain a flavonoid backbone: flavones, flavanones, flavonols, isoflavones, and related condensation products (e.g., coumesterol). The estrogenic activities of diverse phytoestrogenic bioflavonoids and mycotoxins have been extensively investigated in *in vivo* models, *in vitro* cell culture systems, and in ER binding assays, and most of these compounds elicit multiple estrogenic responses in these assays. In addition, a number of plant foodstuffs contain 17β-estradiol (E_2) and estrone (*51,52*).

Environmental and Dietary Antiestrogens

Several different structural classes of chemicals found in the human diet also exhibit antiestrogenic activity (Fig. 2) (*13*). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related halogenated aromatics including polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and PCBs are also an important class of organochlorine pollutants that elicit a diverse spectrum of biochemical and toxic responses (*53*). These chemicals act through the aryl hydrocarbon receptor (AhR)-mediated signal transduction pathway, which is thought to play a role in most of the responses elicited by these compounds. AhR agonists such as TCDD have been characterized as antiestrogens using rodent and cell models similar to those used for determining the estrogenic activity of dietary and environmental chemicals. In the rodent model, TCDD and related compounds inhibit several estrogen-induced uterine responses including increased uterine wet weight, peroxidase activity, cytosolic and nuclear progesterone receptor (PR) and ER binding, epidermal growth factor (EGF) receptor binding, EGF receptor mRNA, and c-*fos* mRNA levels (*54–58*). In parallel studies, the antiestrogenic activities of TCDD and related compounds have also been investigated in several human breast cancer cell lines. For example, structurally diverse AhR agonists inhibit the following E_2-induced responses in MCF-7 human breast cancer cells: post-confluent focus production, secretion of tissue plasminogen activator activity, procathepsin D (52-kDa protein), cathepsin D (34-kDa protein), a 160-kDa protein, PR binding sites, glucose-to-lactate metabolism, pS2 protein levels, and PR, cathepsin D, ER, and pS2 gene expression (*42,59–65*). Moreover, TCDD inhibits formation and/or growth of mammary tumors in athymic nude mice and female Sprague-Dawley rats after long-term feeding studies or initiation with 7,12-dimethylbenzanthracene (*60,66,67*). A recent epidemiology study on women exposed to TCDD after an industrial accident in Seveso (*68*) reported that breast cancer incidence was decreased in areas with high levels of TCDD contamination (particularly in the age class 45 to 74) and among women living longest in an area of low TCDD contamination. Endometrial cancer showed a remarkable decrease, particularly in areas with medium and low TCDD contamination (*68*). Thus, TCDD and related compounds exhibit a broad spectrum of antiestrogenic activities and, not surprisingly, so do other AhR agonists such as the polynuclear aromatic hydrocarbons (PAHs), indole-3-carbinol (IC), and related compounds found in relatively high levels in foodstuffs (*69,70*). PAHs are found in cooked foods (*71,72*) and are ubiquitous environmental contaminants. IC is a major component of cruciferous vegetables (e.g., brussels sprouts, cauli-

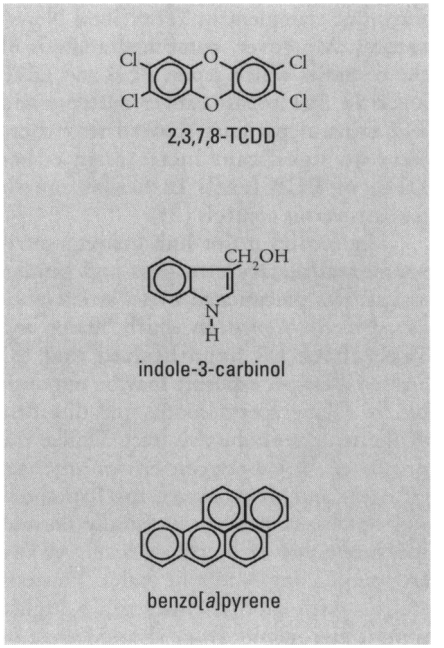

Figure 2. Structures of the environmental and dietary antiestrogens 2,3,7,8-TCDD, indole-3-carbinol, and benzo[*a*]pyrene.

flower) and exhibits antiestrogenic and anticancer (mammary) activities (*70,73*).

Bioflavonoids have been extensively characterized as weak estrogens and therefore may also be active as antiestrogens at lower concentrations. The interaction between estrogenic bioflavonoids and E_2 depends on their relative doses or concentrations, the experimental model, and the specific estrogen-induced endpoint. Markaverich and co-workers (*74*) reported that the estrogenic bioflavonoids quercetin and luteolin (Fig. 1) inhibited E_2-induced proliferation of MCF-7 human breast cancer cells and E_2-induced uterine wet weight increase in 21-day-old female rats. Similar results were also observed in this laboratory for quercetin, resperetin, and naringenin. For example, the bioflavonoid naringenin inhibited estrogen-induced uterine hyptertrophy in female rats and estrogen-induced luciferase activity in MCF-7 cells transfected with an E_2-responsive plasmid construct containing the 5'-promoter region of the pS2 gene and a luciferase reporter gene (unpublished results). In contrast, a recent study (*75*) reported that coumestrol, genistein, and zearalenone were not antiestrogenic in human breast cancer cells. The antiestrogenic activities of weak dietary and environmental estrogens require further investigation; however, it is clear that at subestrogenic doses, some of these compounds exhibit antiestrogenic activities in both *in vivo* and *in vitro* models.

Mass/Potency Balance

The uptake of environmental or dietary chemicals that elicit common biochemical/toxic responses can be estimated by using an equivalency factor approach in which estrogen equivalents (EQs) in any mixture are equal to the sum of the concentration of the individual compounds (EC_i) times their potency (EP_i) relative to an assigned standard such as diethylstilbestrol (DES) or E_2 (*51*). The total EQs in a mixture would be:

$$EQ = \Sigma ([EC_i] \times EP_i)$$

A similar approach is being used to determine the TCDD equivalents (TEQs) of various mixtures containing halogenated hydrocarbons (*76*). Verdeal and Ryan (*51*) have previously used this approach with DES equivalents assuming that the oral potency of E_2 is 15% that of DES. Winter (*77*) has estimated the dietary intake of pesticides based on FDA's total diet study, which includes estimates of food intakes and pesticide residue levels in these foods. The results presented in Table 2 summarize the estimated exposure of different groups to estrogenic pesticides. For example, 14- to 16-year-old males were exposed to a total of 0.0416 µg/kg/day of the estrogenic pesticides, DDT, dieldrin, endosulfan, and *p,p'*-methoxychlor (note: the DDT value represents *p,p'*-DDE and related metabolites, which are primarily nonestrogenic). Thus, the overall dietary intake of these compounds by this age group was 2.5 µg/day.

The relative potencies of dietary and xenoestrogens are highly variable. The results of *in vitro* cell culture studies suggest that estrogenic potencies of bioflavonoids relative to E_2 are 0.001 to 0.0001 (*75,78*) whereas Soto and co-workers (*44*) have assigned an estrogen potency factor of 0.000001 for the estrogenic pesticides. These relative estrogen potency factors for bioflavonoids and pesticides may be lower when derived from *in vivo* studies since pharmacokinetic factors and metabolism may decrease bioavailability. Thus, a more accurate assessment of dietary/environmental EQs requires further data from dietary feeding studies that evaluate these compounds using the same experimental protocols.

The results in Table 3 summarize human exposure to dietary and environmental estrogens and the estimated daily dose in terms of EQs. The relative estrogenic intakes for various hormonal drug therapies were previously estimated by Verdeal and Ryan (*51*); the average estimated daily intake of all flavonoids in food products was 1020 and 1070 mg/day, (winter and summer, respectively) (*52*). The results show that the estimated dietary EQ levels of estrogenic pesticides are 0.0000025 µg/day, whereas the corresponding dietary EQ levels for the bioflavonoids are 102 µg/day. Thus, the EQ values for the dietary intake of flavonoids was 4×10^7 times higher than the daily EQ intake of estrogenic pesticides, illustrating the minimal potential of these industrial estrogens to cause an adverse endocrine-related response in humans.

Previous studies have also shown that AhR agonists, such as TCDD and related compounds, PAHs, and IC and its most active derivative, indolo[3,2-*b*]carbazole (ICZ) all inhibit E_2-induced responses in MCF-7 cells (*59–65,69,70,79*). At a concentration of 10^{-9} M, TCDD inhibits 50–100% of most E_2-induced responses *in vitro* in which the concentration of E_2 is 10^{-9} M. Therefore, 1 TEQ is approximately equal to 1 EQ. The estimated daily intakes of TCDD and related compounds, PAHs, and ICZ (in 100 g brussels sprouts) are summarized in Table 3. The relative potencies of PAHs and ICZ as antiestrogens compared to TCDD are approximately 0.001 in MCF-7 cells (*69,79*). Thus, the TEQs or antiestrogen TEQs can be calculated for the dietary intakes of TCDD and related organochlorines and PAHs (in all foods) (*71,72*). The antiestrogen TEQs for the three classes of dietary AhR agonists are orders of magnitude higher than the estimated dietary intakes of estrogenic pesticide EQs. Thus, the major human intake of endocrine disrupters associated with the estrogen-induced response pathways are naturally occurring estrogens found in foods. Relatively high serum levels of estrogenic bioflavonoids have also been detected in a Japanese male population, whereas lower levels were observed in a Finnish group, and this is consistent with their dietary intakes of these estrogenic compounds (*80*). *p,p'*-DDE is present in

Table 2. Estimated dietary intake of estrogenic pesticides by different age groups based on food intakes and pesticide levels in these foodstuffs (*77*)

	Estimated exposure (µg/kg/day)		
Pesticide	6–11 months	14–16[a] years	60–65 years
DDT (total)	0.077	0.0260	0.0103
Dieldrin	0.0014	0.0016	0.0016
Endosulfan	0.0274	0.0135	0.0210
p,p'-Methoxychlor	0.0005	0.0005	0.0001

[a]Maximum exposure: 60 × 0.0416 = 2.5 µg/day.

Table 3. Estimated mass balance of human exposures to environmental and dietary estrogens and antiestrogens (*51,52,77,79*)

Source	Estrogen equivalents (µg/day)
Estrogens	
Morning after pill	333,500
Birth control pill	16,675
Post-menopausal therapy	3,350
Flavonoids in foods (1,020 mg/day × 0.0001)	102
Environmental organochlorine estrogens (2.5 × 0.000001)	0.0000025
Antiestrogens	TCDD antiestrogen equivalents (µg/day)
TCDD and organochlorines (80–120 pg/day)	0.000080–0.000120[a]
PAHs in food (1.2–5.0 × 106 pg/day; relative potency ~ 0.001)	0.001200–0.0050[b]
Indolo[3,2-*b*]carbazole in 100 g brussels sprouts (0.256–1.28 × 106 pg/day; relative potency ~ 0.001)	0.000250–0.00128[b]

[a]In most studies, 1 nM TCDD inhibits 50–100% of 1 nM E_2-induced responses in MCF-7 cells (*59–65*); therefore, 1 estrogen equivalent ≅ 1 antiestrogen equivalent.
[b]The antiestrogenic potencies of PAHs (*69*) and indolo[3,2-*b*]carbazole (*79*) compared to E_2 were approximately 0.001.

human serum; however, the estrogenic *o,p'*-DDE and *o,p'*-DDT analogs and other weakly estrogenic organochlorine compounds are not routinely detected in serum samples. A recent study identified several hydroxylated PCB congeners in human serum. All of the hydroxylated compounds were also substituted with chlorine groups at both adjacent meta positions (*81*). Based on results of previous structure–activity studies (*43*) for hydroxylated PCBs, these compounds would exhibit minimal estrogenic activity; however, further studies on the activity of hydroxylated PCBs are warranted.

Summary

The hypothesized linkage between dietary/environmental estrogens and the increased incidence of breast cancer is unproven; there is a lack of correlation between higher organochlorine levels in breast cancer patients compared to controls (Table 1) and the low levels of organochlorine EQs in the diet (Table 3). Higher levels of bioflavonoids are unlikely to contribute to increased breast cancer incidence because these compounds and the foods they are associated with tend to exhibit anticarcinogenic activity (*82,83*). The hypothesis that male reproductive problems and decreased sperm counts are related to increased exposure to environmental and dietary estrogens is also unproven. As noted above, dietary exposure to xenoestrogens derived from industrial chemical residues in foods is minimal compared to the daily intake of EQs from naturally occurring bioflavonoids. Moreover, there are serious questions regarding the decreased sperm counts reported by Carlsen and co-workers. Reanalysis of Carlsen et al.'s data suggests that there has not been a decrease in sperm counts in males over the past 30 years (*33*) and possibly over the past 50 years (*34*). Thus, in response to articles in the popular and scientific press such as "The Estrogen Complex" (*7*) and "Ecocancers: Do Environmental Factors Underlie a Breast Cancer Epidemic?" (*8*), the results would suggest that the linkage between dietary or environmental estrogenic compounds and breast cancer has not been made, and further research is required to determine the factors associated with the increasing incidence of this disease.

Note added in proof. A recent study (*84*) reported a 2.1% decrease in sperm concentrations in France from 1973 to 1979.

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