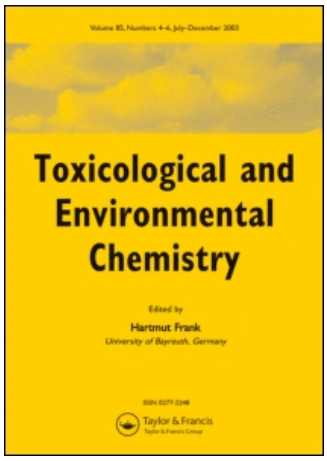

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Human exposure to medicinal, dietary, and environmental estrogens

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Abstract

Estrogens are a broad class of compounds which exert many physiological effects. In addition to gonadal and peripheral endogenous estrogen production, humans can be exposed to exogenous estrogenic compounds (xenoestrogens) from medicinal, dietary and environmental sources. These compounds can exert effects similar to that of 17β-estradiol (E2) through estrogen receptor (ER)-mediated mechanisms, or via ER-independent pathways. Although estrogens are used for a number of medical purposes such as birth control and for hormone replacement therapy (HRT) in the treatment of post-menopausal symptoms, studies have found adverse health effects from their use. Increased risk of stroke and invasive breast cancer are associated with medicinal estrogen use. As an alternative to HRT, diets rich in phytoestrogens are used by many women. Even though phytoestrogen consumption is associated with reduced risk of hormone-dependent cancers and antioxidant properties, concerns about adverse effects, such as endocrine disruption, cannot be dismissed. Widespread use of chemicals with estrogenic properties in agriculture and industry has resulted in endocrine disruption in wildlife populations although the impact on human health is still in debate. In general, the ranking order of estrogen equivalent factors compared to E2 is medicinal estrogens > phytoestrogens > environmental estrogens. Serum concentrations of estrogens vary among populations depending on choice of diet, use of medicinal estrogens, and environmental exposure. Thus, determination of total exposure levels (i.e. E2 equivalent concentration) is complex and can vary greatly within a larger population.

Keywords: *Medicinal estrogens, phytoestrogens, environmental estrogens, public health, exposure level*

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Existence of a variety of estrogens

Estrogens are a broad class of compounds which exert many physiological effects. Included in this group are endogenous sex steroid hormones such as the potent 17β-estradiol (E2), which has a major role in secondary sex organ development, behavior, and fertility. E2 is produced primarily by the ovarian follicle in pre-menopausal women. In post-menopausal women, low levels of the endogenous estrogens E2, estrone (E1), and the biologically inactive estrone sulfate are produced peripherally [1].

In addition to endogenous production, humans can be exposed to a variety of exogenous estrogens (xenoestrogens) via medical treatment, dietary consumption, and environmental exposure. Medicinal estrogens are used for many purposes including birth control, reduction of post-menopausal symptoms, and treatment of endometrial cancer [2]. The synthetic estrogen ethinyl estradiol is used in the majority of oral contraceptives [3]. Other estrogens used medically are equilin, equilenin, 17α- and 17β-dihydroequilin (Table I). Hormone replacement therapy (HRT) for post-menopausal symptoms is one of the most prevalent uses of medicinal estrogens. Estrogenic compounds used in HRT can include natural sources such as conjugated equine estrogens extracted from the urine of pregnant mares and synthetic estrogens. At least 10 estrogens have been identified in conjugated equine estrogens including E2, 17α-estradiol, E1, equilenin, and equilin [4].

Other natural occurring sources of estrogenic chemicals are plants and microbes. The phytoestrogens are a broad group of plant-derived or microbial compounds that are structurally or functionally similar to E2. Examples include genistein and coumestrol which have agonistic effects to E2 [5]. Phytoestrogens are non-steroidal compounds which exert a number of estrogenic effects [5,6]. The exact mechanism(s) by which phytoestrogens exert effects similar to E2 has not been elucidated.

Another source of estrogenic compounds to which humans are exposed is industrial or agricultural activity. Endocrine-disrupting chemicals (EDCs) are defined as ''exogenous agents that interfere with the production, release, transportation, binding, action, or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of the developmental processes'' [7]. According to the above definition, EDCs may interfere with physiological functions of a variety of hormones such as estrogens, androgens, or thyroid hormones. In this article we focus on the environmental chemicals that possess activity of E2, namely estrogenic endocrine-disrupting chemicals (EEDCs). Polychlorinated biphenyls (PCBs) are one such class of compounds. PCBs were used in the manufacturing of electrical equipment, and have since been linked to adverse reproductive effects [8]. It has been proposed that excess exposure to estrogens may lead to endocrine disruption, which has been manifested in numerous wildlife studies [9,10]. Whether EEDCs cause adverse effects in humans is still debated.

Estrogen synthesis, metabolism, and physiological function

The hypothalamic-pituitary-gonadal axis controls the gonadal production and release of estrogens (Figure 1). Possible targets of exogenous estrogens thus include the hypothalamic-pituitary-gonadal axis, E2 synthesis, metabolism, secretion, transport, and signal transduction. Estrogen's primary source in normally cycling adult women is the ovarian follicle which secretes varying levels of E2 depending on the phase of the menstrual cycle [11]. Release of gonadotrophin-releasing hormone (GnRH) by the hypothalamus stimulates the anterior pituitary to produce two hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [12]. In response to LH, follicular

Table I. Exposure of medicinal estrogens and the steady-state concentrations in plasma. Italicized numbers in "Dosage available" column correspond to the numbers in "Serum level" column [116–121].

Brand name (form)	Types of estrogens	Dosage available (mg)	Serum level (ng mL^{-1})
Premarin® (tablet)	Estrone, equilin, 17α- and 17β-dihydroequilin, 17α- and 17β-estradiol	0.3, *0.625* (*2x*), 0.9, 1.25, 2.5	12.3
Prempro® (tablet)	Conjugated estrogens found in premarin, medroxyprogesterone	0.625 (*2x*)	11.7
Cenestin® (tablet)	Conjugated estrogens found in premarin tablets, 17β-estradiol, equilenin, 17α- and 17β-dihydroequilenin	0.3, *0.625* (*2x*), 0.9, 1.25	7.7
Alora® (transdermal patch)	17β-Estradiol	*0.05*, *0.075*, *0.1*	0.092, 0.120, 0.144
Climara® (tablet)	17β-Estradiol	0.025, *0.05*, *0.075*, *0.1*	0.032, 0.071, 0.147
Vivelle® (transdermal patch)	17α-Ethinyl estradiol	0.025, *0.0375*, *0.05*, *0.075*, *0.1* (*1x*)	0.046, 0.083, 0.099, 0.133

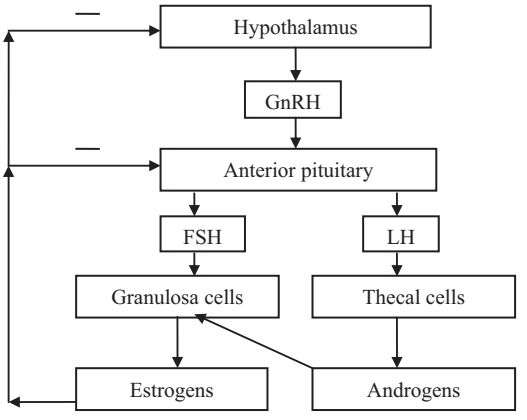

Figure 1. Estrogen metabolism is under the control of the hypothalamic-pituitary-gonadal axis.

thecal cells produce the androgens testosterone and androstenedione which are precursors to estrogen synthesis [12]. Androstenedione and testosterone diffuse into follicular granulosa cells, where FSH stimulates these cells to aromatize the two androgens to the estrogenic steroids estrone and estradiol, respectively [12–14]. 17β-Hydroxysteroid dehydrogenase and aromatase catalyze conversion of androgens to estrogens. Within the granulosa cells of pre-ovulatory follicles, higher levels of the enzyme aromatase which converts androgens to estrogens are present than thecal cells [13]. During the luteal phase of the ovarian cycle, the corpus luteum of primates secretes estrogens. This two-cell model of estrogen synthesis with thecal cells of the corpus luteum producing androgens that are aromatized to estrogens by granulosa-derived cells is supported by steroidogenic enzyme localization [15].

Following menopause, most endogenous estrogens are produced by conversion of androstenedione, secreted by the adrenal cortex, to estrone by peripheral tissues [16]. The most abundant circulating estrogens in post-menopausal women are estrone and the inactive sulfate-conjugated form, estrone sulfate. Peripherally, estrone sulfate can be converted by steroid (estrone) sulfatase to the biologically active estrone [1].

Mechanisms of estrogenic effects

Compounds may exert their estrogenic activities via estrogen receptor (ER)-independent or dependent mechanisms. Two forms of human ERs (ERα and ERβ) have been identified [17–22]. These two receptors encoded by two different genes (ESR1 and ESR2) have distinct tissue expression patterns [23]. For both receptors, many mRNA splice variants exist with differing affinities to the same compound [24]. For a comprehensive review on ERs and human diseases, readers are referred to other review papers [25,26].

Figure 2 depicts the classical ER-mediated signaling pathway. The binding of E2 or other estrogenic ligands to the ER causes a conformational change in ER protein structure and dissociation of the heat shock protein 90 (hsp90). The ligand-receptor complex then undergoes homodimerization and the resulting homodimer complex binds to estrogen response element (ERE) of the genes that are estrogen-inducible. Once bound to ERE, the ER homodimer may induce or inhibit gene expression; consequently, homeostasis of an organism might be altered.

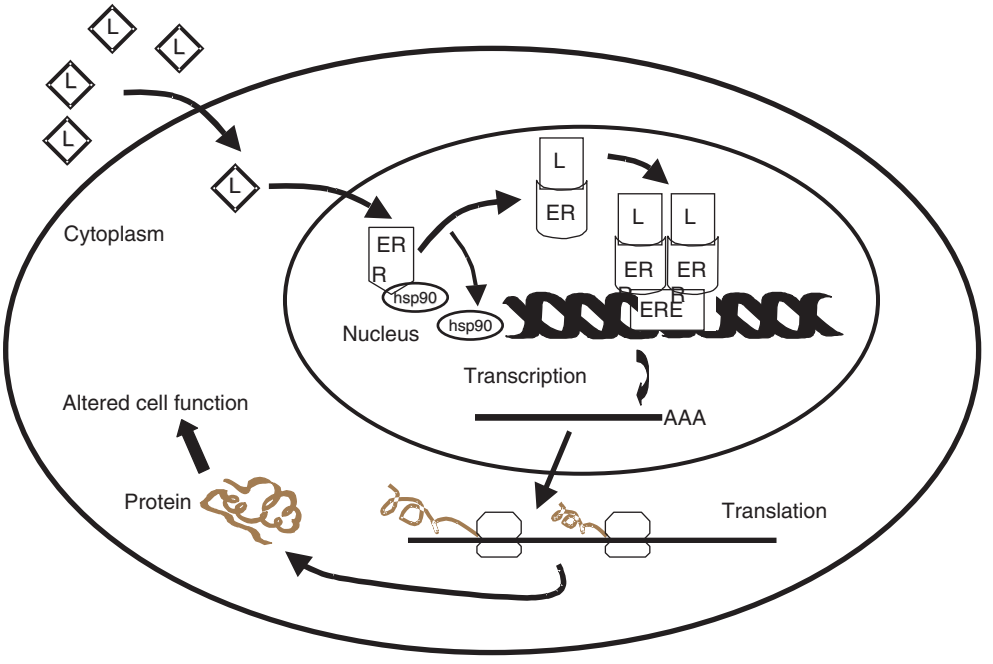

Figure 2. Estrogen receptor-dependent mechanism of action of estrogenic chemicals. "L" denotes endogenous or environmental ligands. Details are described in the text.

In addition to the classical mechanism, estrogens can act either through the ER located in or adjacent to the plasma membrane, or through non-ER membrane-associated estrogen-binding proteins. These non-genomic actions contribute to rapid effects of estrogens by activating the mitogen-activated protein kinase (MAPK) and the phospho-inositol 3-kinase signal pathway, stimulating adenylate cyclase activity and cAMP production, and mobilizing intracellular calcium [27–39].

In addition to acting via non-ER membrane-associated estrogen-binding proteins, estrogenic chemicals may also exert physiological functions via other ER-independent pathways or by alterations of cellular and extra-cellular components. For instance, 2-chloro-s-triazine herbicides such as atrazine, simazine, and propazine are capable of inducing aromatase (CYP19) which converts androgens to estrogens [40]. Another indirect mechanism is competition among environmental chemicals for steroid hormone binding globulins, which alters cellular availability of a specific chemical. Figure 3 further illustrates multifaceted interactions which include metabolic pathways, steroidogenesis, ligand transport, and receptor crosstalks. These factors need to be taken into account when one tries to characterize mechanisms of action of exogenous estrogens.

Routes of exposure and bioavailability of estrogens

Humans can be exposed to natural or synthetic estrogens from many sources. For example, synthetic estrogens from industry or agriculture may enter into the environment via point or non-point source discharges, or incidental spills resulting in contamination of ground water

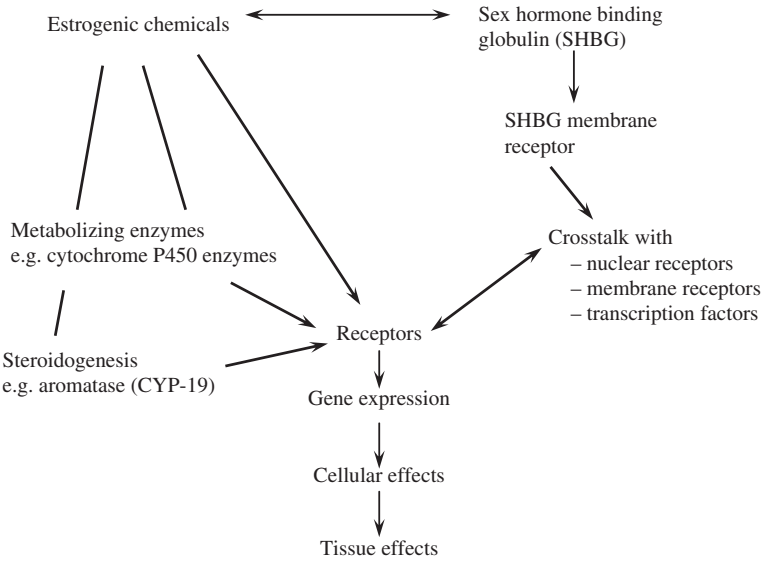

Figure 3. Crosstalks among several physiological pathways and mechanisms of actions complicate prediction of risk assessment for estrogens.

and drinking water. In addition, phytoestrogens are included in the staple diet of some cultures. Therefore, humans may contact natural and synthetic estrogenic chemicals via dietary consumption or contamination, medicinal and personal care product administration, and environmental exposure. These three sources of estrogens are further described as follows.

Medicinal products containing estrogenic chemicals

Medicinal estrogens are used for a number of purposes. Estrogens, in combination with progestin, are used for birth control and treatment of endometriosis [2]. Estrogens can be administered orally in tablet form or by a newer method of transdermal patches. Medicinal estrogens are also used to treat post-menopausal conditions such as vasomotor symptoms, vaginal atrophy and dryness, and osteoporosis. Vasomotor symptoms associated with menopause include hot flashes and night sweats. For moderate to severe menopausal vasomotor symptoms, HRT decreases the frequency and severity of symptoms [41]. Estrogens are also important in bone deposition and are required to maintain adequate bone density [42]. Studies have found that an additional benefit of HRT is the reduction of risk of skeletal fractures [43].

The dosage of medicinal estrogen prescribed varies with a patient's needs. Table I lists commonly prescribed estrogens, available dosages, and steady-state concentrations in serum. These estrogens are either synthetic chemicals or extracts from animals such as conjugated equine estrogens (Premarin®) from horse urine. Serum medicinal estrogen concentrations range between 0.032 and 12.3 $\mathrm{ng\,mL^{-1}}$. Our statistical analysis indicated that serum steady state concentrations of individual medicinal estrogens significantly correlates with their respective dosages ($R^2 = 0.89$). In general, the order of the estrogenic potency, measured as cell proliferation, is 17α-ethinyl estradiol = E2 > estriol > estrone (Table II).

Table II. Relative receptor binding affinity and gene expression among phytoestrogens, medicinal estrogens, and environmental estrogens. E2 was used as the comparative standard (100% receptor binding affinity and transactivation EC_{50} ratio, cell proliferation equivalence factor = 1).

Chemicals	Receptor binding RBA[a] (%)	Transactivation EC_{50} ratio[b] (%)	Cell proliferation equivalence factor[c] ($\times 10^{-6}$)
Phytochemicals			
Isoflavone			
Formononetin	–	0.0486	–
Daidzein	–	0.0052	110 [122]
Biochanin A	–	0.0618	12 [123]
Genistein	0.46	0.0773	260 [122]
Flavonoid			
Quercetin	wb	–	1 [60]
Kaempferol	–	–	70 [122]
Luteolin	–	–	59 [122]
Apigenin	–	–	150 [122]
Naringenin	wb	0.0003	77 [122]
Phloretin	–	–	25 [122]
Coumestan			
Coumestrol	0.81	0.0156	10 [124]
Resorcylic acid lactones			
α-Zearalenol	48	12.180	10^4 [125]
β-Zearalenol	13	1.3571	–
Zearalenone	9.3	–	10^4 [124]
Lignans			
Enterolactone	–	–	1 [126]
Enterodiol	–	–	0.1 [126]
Medicinal estrogens/antiestrogens			
17α-Ethynyl estradiol	127	–	10^6 [124]
Estrone	45	–	10^4 [124]
Estriol	28	–	10^5 [124]
Tamoxifen	11	–	10 [124]
Environmental chemicals			
DDT and metabolites	–	–	1 [124]
Bisphenol A	0.0080	0.0035	10^3 [127]
o,*p*′-DDT, *p*,*p*′-DDT	wb	wi	1 [125]
PCBs, OH-PCBs, Aroclor mixtures	nb[d]	wi[d]	1–10^2 [124]

[a]Relative binding affinity (RBA) = (IC_{50} E2/IC_{50} compound) × 100. Experiments were carried out using a human recombinant ER-α (GST-hERαdef) [128,129]. wb = weak binder, nb = non-binder.
[b]Isoflavones were tested with a yeast estrogen screen system [130]. Other EC_{50} values were determined using a human recombinant ER-α (GST-hERαdef) and a luciferase reporter gene [129]. wi = weak inducer.
[c]Equivalence factors were relative comparisons against E2.
[d]Data from [131,132].

Although medicinal estrogens may provide health benefits, studies have also reported that these compounds can cause adverse biological effects. In the classic example of diethylstilbestrol (DES) which was given to women to prevent spontaneous abortions in the United States from the late 1940s until 1971, prenatal exposure to DES caused negative health effects in offspring. Daughters born to these women had increased risk of vaginal

clear cell carcinoma and a higher proportion of adverse reproductive outcomes for first pregnancies [44,45]. More recently, evidence supports use of estrogens and increased endometrial cancer risk [46]. Preliminary analysis from the long-term Women's Health Initiative studies reported increased risks of myocardial infarction, stroke, invasive breast cancer, pulmonary emboli, and deep vein thrombosis in post-menopausal women during 5 years of treatment with conjugated equine estrogens combined with medroxyprogesterone acetate [43]. Whether the medical benefits outweigh the risks is still under rigorous investigation. Subjective and personal factors will also influence a woman's decision to use medicinal estrogens.

Dietary consumption of phytoestrogens

Phytoestrogens can occur naturally in the diet or be consumed as dietary supplements. Sources of phytoestrogens include soybeans, clover, alfalfa sprouts, oilseeds, whole grains, fruits, and vegetables. Phytoestrogen consumption is associated with positive effects on total blood cholesterol and low density lipoprotein, cholesterol levels [47]. Studies have also found an association between phytoestrogen intake and induction of apoptosis, antioxidant effects, and inhibition of DNA topoisomerase [47–54].

Evidence from epidemiological studies on humans and laboratory experiments on mammalian species indicate that consumption of phytoestrogen-rich food is correlated with a number of health benefits including reductions in breast cancer risk, osteoporosis, menopausal discomfort, cardiovascular disorders, and bone diseases [55]. For instance, ipriflavone (a phytoestrogen derivative) and coumestrol have both demonstrated protection against bone density loss in post-menopausal women [42]. Because known health risks such as increased breast cancer risk and stroke are associated with the use of medicinal estrogens, and the effects of long-term treatment with these compounds are also unknown, women may elect to take natural alternatives to conventional HRT [43]. However, studies relating to menopausal vasomotor symptoms and phytoestrogens are inconclusive. Relief of hot flashes and vaginal dryness by women including soy and flaxseed in their diet has been reported [55]. At this time, sufficient clinical evidence is not available to determine if soy foods or isoflavone supplements affect menopause-associated vasomotor symptoms [41]. Evidence for the estrogenic effects of phytoestrogens is supported by experiments with aromatase knockout mice (ArKO) which lack the ability to convert androgens to estrogens. Adverse reproductive effects such as ovarian follicular abnormalities and decreased spermatogenesis in ArKO mice fed phytoestrogen rich diets were attenuated compared to ArKO mice on a phytoestrogen free diet [5,56].

Epidemiological evidence suggests that hormone-dependent cancers, such as breast and prostate cancers, are statistically less prevalent in Asian countries than in the USA and Western Europe [55]. Greater consumption of phytoestrogen-containing foods, such as soy, by Asians is thought to be a reason for this difference. The mechanisms by which phytoestrogens could exert a chemopreventive influence are multiple and not clearly understood. Animal studies have demonstrated that neonatal exposure to the phytoestrogen genistein may protect against chemically induced tumor multiplicity [57,58]. Other studies have found a decrease in tumor multiplicity associated with a diet of soy protein containing isoflavones [59]. Additionally, genistein has also been shown to inhibit angiogenesis and proliferation of endothelial cells [55]. Antioxidant effects of phytoestrogens may also be related to the anticarcinogenic affects of these plant-derived compounds [53]. Studies with MCF-7 human breast cancer cells suggest that the antiproliferative effects of flavonoids are independent of ER mechanisms [60]. Flavones and isoflavones are competitive inhibitors

Table III. Estimated dietary exposure (mg day^{-1}) to environmental estrogens and phytoestrogens in different countries.

Chemicals	USA (mean ± SD)	New Zealand (ranges)	UK (mean ± SD)	Japan (mean ± SD)
Daidzein	0.289 ± 2.104	0.87–1.2	0.314–0.405	16.4 ± 7.5
Genistein	0.338 ± 2.119	1.2–1.9	0.389–0.499	31.3 ± 14.4
Total isoflavones	0.760 ± 4.345[a]	2.07–3.1[b]	0.718–1.041[c]	47.7[d]
Secoisolariciresinol	0.622 ± 0.357	–	–	–
Enterolactone	–	0.19–0.28	–	–
Matairesinol	0.023 ± 0.019	–	–	–
Enterodiol	–	0.1–0.16	–	–
Total lignans	0.645 ± 0.363[a]	0.29–0.44[b]	–	–
Total environmental estrogenic chemicals[e]	0.0025[e]	0.0036–0.0073[b]	–	–

[a]Post-menopausal women in the Framingham Offspring Study [133].
[b]Ranges of the averages from different genders and age groups [134].
[c]Data were from the VENUS database [135].
[d]Japanese women [136].
[e]Major estrogenic pesticides include DDT and metabolites, dieldrin, endosulfan, and *p,p′*-methoxychlor [137].

of cytochrome p450 aromatase, the enzyme complex responsible for converting androgens to estrogens resulting in lowering of estrogen levels in peripheral and/or cancer cells [61]. Consequently, they may play a protective role as antipromotional compounds during growth of estrogen-dependent cancers [62].

Although most of the mechanisms of action of the above biological effects still remain largely unknown, studies have shown that phytoestrogens are involved in estrogen metabolism pathway, sex hormone binding globulin regulation, and cell cycle alteration [63–68]. For instance, phytoestrogens (e.g., flavonoids, coumarins, coumestans) influence reductive and oxidative activities of 17β-hydroxysteroid dehydrogenase type 5 and 17β-hydroxysteroid dehydrogenase type 1, which result in altered estrogen metabolism pathway [69,70].

Tables III and IV summarize the estimated dietary intake of environmental estrogens and phytoestrogens and their serum levels for populations in the USA, New Zealand, UK, and Japan. As indicated in the tables, the Japanese population is exposed to high phytoestrogen levels. Average dietary intakes of total isoflavones in the USA, New Zealand, and the UK are between 1 and 3 mg day^{-1}. On the other hand, intake of isoflavones in Japanese women can be as high as 47.7 mg day^{-1}. This is not surprising because the traditional Japanese diet contains significant amounts of legume products. Overall, the intake and serum phytoestrogen concentrations are highly variable within and among populations. Additional studies should address the lack of estimated dietary exposure and serum levels of lignans in the UK and Japan to allow more accurate comparison of phytoestrogen consumption and serum concentrations. Infants who are fed soy-based formulas because of either lactose or milk protein intolerance constitute another population with high phytoestrogen exposure [71,72]. Total isoflavones serum concentrations of 4-month old infants fed soy-based formula can be 100 times higher than infants fed with milk-based formula (981 *vs.* 9.4 ng mL^{-1}) [72]. Post-menopausal women who elect to take phytoestrogen-containing products as a ''natural alternative'' to HRT are a third population

Table IV. Levels (ng mL^{-1}) of phytoestrogens and environmental estrogens detected in human serum or plasma.

Chemicals	USA[a]	New Zealand[b]	UK[c]	Japan[d]
Daidzein	nd–162 (3.9)	28.4–30.1	0.1–44.8 (2.0)	71.8, 62.7
Equol	nd–8.2 (LOD)	–	nd–8.0 (0.2)	23.7, 13.8
Genistein	nd–203 (4.7)	83.1–110	nd–159.7 (4.1)	133.1, 135.6
Total isoflavones	nd–373.2 (8.6)	111.5–140.1	nd–212.5	209.6, 231.1
Enterolactone	nd–112 (3.6)	5.4–7.0	nd–388.1 (3.8)	9.72, 6.77
Matairesinol	nd–3.3 (LOD)	–	–	–
Enterodiol	nd–19 (1.8)	0.46	nd–8.1 (0.4)	–
Total lignans	nd–134.3 (5.4)	0.46–5.7	nd–396.2	9.72, 6.77
Total environmental estrogenic chemicals	2.39–6.85[e]	0.06–0.44	0.01–13.39[f]	–

Ranges are given followed by means in parentheses. nd = not detectable.
[a]The Third National Health and Nutrition Examination Survey from 1988–1994 [138].
[b]New Zealand males [134].
[c]Subjects from the European Prospective Investigation of Cancer and Nutrition-Norfolk Study (1993–1997) UK females [139].
[d]Means were taken from males and females, and placed in the order of ♂, ♀ [140].
[e]DDT and metabolites, aldrin, dieldrin, and *p,p′*-methoxychlor in the control subjects of the Long Island Breast Cancer Study Project [141]. The ranges of the data were within one standard deviation.
[f]DDT and metabolites from volunteers age 18 and over [142].

with high exposure to phytoestrogens [73]. Total isoflavone levels of 32 commercially available supplements are variable with a range from 2.3 to 48.8 mg day^{-1} [74].

Although phytoestrogens may have physiological benefits, concerns exist about possible adverse biological effects, namely endocrine disruption. In the past, it has been proposed that development of infants might be affected by consumption of estrogenic compounds in the milk. No epidemiological evidence has substantiated effects of soy-based formula on growth rate, age to reach menarche, or infertility for infant populations who consumed soy-based formula [71]. Although phytoestrogens such as genistein possessed high affinity to both mouse and human ERs, mice exposed to genistein at human consumption levels did not experience adverse effects on sperm quality [75]. Still, additional studies need to be conducted to gain further understanding regarding the possible effects of phytoestrogens on reproductive functions. These studies are crucial because of the complexity of the signal transduction pathways, tissue-specific responses, and the involvement of a variety of genes which possess ERE in the promotion region (Figures 2 and 3).

Environmental estrogenic chemicals

Environmental estrogens include herbicides and pesticides from residential and agricultural runoff and industrial chemicals from the manufacture of paper, paint, and plastic products [76–82]. Many commonly used pesticides such as the triazines and pyrethroids demonstrate estrogenic properties [83,84]. In fact, a review of 48 EDCs found that estrogenic effects were demonstrated predominately in pesticides [85]. Exposure to environmental estrogens has been associated with abnormalities in reproductive organs and malformations in wildlife [9,76,86]. Studies have shown that male fish held in treated sewage effluents exhibit increased levels of vitellogenin, an indication of exposure to estrogens [87–90]. Environmental estrogens such as alkylphenol polyethoxylates (APEOs) were commonly present in many streams in Europe, and male fish held downstream of sewage treatment plants or treated with environmentally relevant concentrations of APEOs exhibited

increased levels of vitellogenin or modified gonadosomatic indices [89–93]. Transport of estrogenic PCBs to the Arctic region via air and ocean currents has led to bioaccumulation, resulting in increases in progesterone levels in females [94]. This prevalence of estrogenic compounds and evidence of endocrine disruption in wildlife prompted the US Congress to make amendments to the Food Quality Protection Act (PL 104-170) and the Safe Drinking Water Act (PL 104-182) in 1996, requiring the US Environmental Protection Agency (USEPA) to screen and test for chemicals that might mimic activities of estrogen. The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), an advisory group to the USEPA, was formed to provide recommendations to comply with congressional mandates. To date, voluminous studies on endocrine disruption have been conducted in laboratory and field.

Humans may be exposed to environmental estrogens via food and water consumption, inhalation, or direct skin contact. In general, the exposure levels of environmental estrogens and their serum concentrations were lower than those of phytoestrogens (Tables III and IV). No human health benefits have been reported from exposure to environmental estrogens. Whether exposure to environmental estrogens is associated with cancer incidence or other adverse effects on human health is debated. For instance, two studies found an association between triazine exposure and estrogen-dependent cancers [84,95]. In contrast, a review of eight epidemiologic studies concluded that there was limited support for an association between atrazine exposure and several cancers [96].

To assess the risk of human exposure to environmental estrogens, many studies have been conducted with surrogate mammalian species. Comparisons among studies are difficult to make due to the variations in species or strains tested, exposure duration, and toxic end points. Nevertheless, a number of studies have demonstrated that estrogenic chemicals are capable of eliciting a wide spectrum of adverse effects including reduced ovarian and uterine weights, delayed or early vaginal opening, disrupted or irregular estrous cyclicity, reduced fecundity, and suppressed LH secretion [79,97–100]. These results confirm the general thought that critical developmental stages of urogenital tract *in-utero* and during early post-natal life are particularly sensitive to hormonal disruption. In humans, the consumption of rice contaminated with PCBs in the late 1970s by pregnant Taiwanese women demonstrated the effects of these compounds on pre-natal development. Boys exposed pre-natally to PCBs and their heat degradation products exhibited significantly elevated levels of E2 when compared with controls. At puberty, the exposed boys had decreases in serum testosterone and increases in serum FSH levels [101]. Several factors need to be considered when comparisons are made between animal data and human exposure risk. First, most of the laboratory studies were conducted with a single chemical or dosages that were typically much higher than environmental exposure levels. The response pattern of high dosages might be different from that of environmentally relevant dosages due to intricate physiological, pharmacokinetic, and pharmacodynamic processes. Second, environmental exposure usually involves multiple chemicals that might act via a variety of signal transduction pathways and these pathways might crosstalk with each other. A third factor to consider is that agonists and antagonists may co-exist in a mixture and biological effects by the mixture may be synergistic, additive, or attenuated. For instance, tetrachlorodibenzo-*p*-dioxin (TCDD) is considered antiestrogenic and in many cases it coexists with polychlorinated bisphenols (PCBs) and other compounds of weak estrogenicity [102–104]. Additionally, species-dependent sensitivity of animal models and toxic end points relevant to human health need to be taken into consideration when data is to be used for risk assessment. Thus, extrapolation of these data to assess human exposure risk becomes difficult and complicated.

Issues and considerations related to calculation of mass and potency balance

One may attempt to calculate the respective total EEQs (17β-estradiol equivalent concentration) of medicinal, dietary, and environmental estrogens to evaluate the degree of exposure by summing the EEQs of each individual chemical. An equation to calculate total (EEQ) for each category of estrogens has been proposed as follows [105].

$$\text{Total EEQ} = \sum(\{\text{SC}_i\} \times \text{EF}_i)$$

SC_i = serum concentration of chemical i, whereas EF_i = equivalent factor of chemical i relative to E2 in cell proliferation.

A variety of issues and difficulties immediately arise in this simplified model. A deficiency of data that can be used to calculate EEQs exists. Although Table II is probably the most comprehensive list of EF_i from cell proliferation assays, thousands of suspected estrogenic chemicals remain untested. Even though cell proliferation tests are highly sensitive, accurate, and generate data that is more available than receptor binding and gene expression tests data, a calculation model built upon it may be oversimplified. Cell proliferation tests do not take into account the pharmacokinetics and pharmadynamics of chemicals. Thus, it is desirable to include more physiological and toxicological end points from whole animal studies to make it more relevant in assessing the impacts on human health. Another difficulty encountered at the present is that most of the estrogens' steady-state serum concentrations are relatively unknown, as evident in Table IV. An intensive search of the literature only produced a few of the concentrations which had been tested because of medical or nutritional interest. All these reasons make an accurate prediction of human exposure to these three categories of estrogens impossible at this stage.

Although difficulties exist in quantitative determination of EEQs, the trends in exposure to these three categories of estrogens are not difficult to observe. In general, the EFs of medicinal estrogens are high, ranging from 1 to 100% in comparison with E2. On the other hand, except for bisphenol A, the EFs of phytoestrogens and environmental chemicals are relatively low, ranging from 0.00001 to 0.03%, in comparison with E2. The general ranking order of EFs is medicinal estrogens > phytoestrogens > environmental estrogens. Prescribed medicinal estrogen exposure in women should be similar in the four countries. Total isoflavone exposure is highest in Japan followed by New Zealand, with much lower exposures in the USA and UK, according to the best available data. In general, due to the very low estrogenicity based upon cell proliferation data and their low serum concentrations, the predictive EEQ for environmental estrogens should be considered the lowest among these three categories, assuming most of the untested chemicals possess relatively low biological activities. However, the predictive low EEQ of environmental estrogens should not be interpreted to imply that these compounds have a negligible impact on human health, and continued surveillance of health effects of environmental estrogens is critical.

Future research areas

The future research for environmental estrogens has been described in previous sections. Here we focus on research need in medicinal and dietary estrogens. The use of medicinal estrogens to alleviate post-menopausal symptoms must be considered in light of results of the Women's Health Initiative trial. Reduced risk of osteoporosis and dementia and relief of post-menopausal symptoms associated with HRT must be weighed against the increased risk of stroke, coronary heart disease, venous thromboembolism, and breast cancer found in

the Women's Health Initiative study [43,106]. Additionally, using only estrogens in HRT increases the risk of endometrial cancer [46]. The relationship between cardiovascular disease and HRT was one of the least expected findings of the Women's Health Initiative trial. Prior to the study, HRT was thought to provide a protective effect for coronary heart disease. Instead, the relative risk of coronary heart disease for women taking HRT was increased [43]. Because risk factors for coronary heart disease such as hypertension, elevated plasma cholesterol, and elevated triglycerides, increase with menopause [107], preventing coronary heart disease in post-menopausal women is an urgently needed research focus. The results of the Women's Health Initiative study must be reconciled with other studies to understand the health outcomes of medicinal estrogens for post-menopausal women. Elucidating the relationship between medicinal estrogens and health effects in post-menopausal women must be the primary objective for future investigation.

An additional research focus for medicinal estrogens is the development of pharmaceuticals that provide the benefits of HRT without the adverse affects. Furthermore, determining the dosages and durations of HRT which provide the most health benefits with the fewest risks is an area of continuous investigation. Thus, development of new regimens of HRT is an area of intense basic/clinical research. At the present, tibolone, a synthetic chemical with estrogenic, progestogenic, and androgenic properties, provides relief of vasomotor symptoms and prevention and treatment of osteoporosis, but whether it provides the same risks as other regimens of HRT has not been determined [43,46]. Another compound, raloxifene, is a member of the Selective Estrogen Receptor Modulator (SERMs) class of drugs which can have either agonistic or antagonistic affects on ERs. Acting as antiestrogen in the breast, raloxifene causes a reduction in breast cancer incidence, and prevents osteoporotic vertebral fractures but not hip fracture and does not relieve vasomotor symptoms [43]. Tamoxifen, a SERM, acts as an antiestrogen in ER-positive cells and is used as an adjuvant to prevent breast cancer reoccurrence in patients [108]. Letrozole (Femara®), an aromatase inhibitor is used in a wide range of breast cancer settings. Letrozole has greater efficacy than tamoxifen in randomized clinical trials in post-menopausal women with hormone-responsive early-stage breast cancer [109,110]. Assessing the health effects of HRT regimens like tibolone and raloxifene or a combination of medicinal estrogens is a continuing research need. Medicinal estrogens can be given to women having severe menopausal symptoms while for milder symptoms phytoestrogens (i.e., isoflavones) can be an alternative [106].

Another area of needed research concerns the health effects of dietary estrogens. The increased risk of breast and prostate cancers among Asian immigrants in the US was assumed to be related to cancer-causing agents such as fats in the western diet. However, the Nurses' Health Study did not substantiate this hypothesis [111,112]. Recent attention has been directed to the study of cancer-preventing constituents in the oriental diet. Messina et al. found that approximately two-thirds of the reported studies (16/27) correlated soy intake with a reduction of cancer risk [113]. Studies have indicated that isoflavones such as genistein and daidzein in soy products are correlated with health benefits such as reduced cancer incidence [55]. However, a recent study showed no significant difference in reduction of tumor multiplicity in adult female rats (all approximately at 50% level) between soy protein isolate depleted of isoflavones (genistein and daidzein) and soy protein isolate containing normal levels of isoflavones [59]. This suggested that some unknown factors in soy protein isolate may be as important as isoflavones in suppressing DMBA-induced mammary carcinogenesis. Additional studies are needed to identify and characterize the unknown factors. It has also long been hypothesized that exposure to

phytoestrogens in early life may have long-term health benefits for hormone-dependent diseases, although specific mechanisms of action are still largely unknown [114,115]. Accordingly, the hormonal and non-hormonal roles and the cancer-preventing constituent(s) in complex soy constituents deserve further investigation. Due to the possibility of self administration of large doses, studies on possible adverse effects by phytoestrogens need to be continued to ensure safety of dietary supplements.

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