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An Updated Review of Environmental Estrogen and Androgen Mimics and Antagonists

Carlos Sonnenschein* and Ana M. Soto

Tufts University School of Medicine, Department of Anatomy and Cellular Biology, 136 Harrison Ave, Boston, MA 02111, U.S.A.

For the last 40 y, substantial evidence has surfaced on the hormone-like effects of environmental chemicals such as pesticides and industrial chemicals in wildlife and humans. The endocrine and reproductive effects of these chemicals are believed to be due to their ability to: (1) mimic the effect of endogenous hormones, (2) antagonize the effect of endogenous hormones, (3) disrupt the synthesis and metabolism of endogenous hormones, and (4) disrupt the synthesis and metabolism of hormone receptors. The discovery of hormone-like activity of these chemicals occurred long after they were released into the environment. Aviation crop dusters handling DDT were found to have reduced sperm counts, and workers at a plant producing the insecticide kepone were reported to have lost their libido, became impotent and had low sperm counts. Subsequently, experiments conducted in lab animals demonstrated unambiguously the estrogenic activity of these pesticides. Man-made compounds used in the manufacture of plastics were accidentally found to be estrogenic because they fouled experiments conducted in laboratories studying natural estrogens. For example, polystyrene tubes released nonylphenol, and polycarbonate flasks released bisphenol-A. Alkylphenols are used in the synthesis of detergents (alkylphenol polyethoxylates) and as antioxidants. These detergents are not estrogenic; however, upon degradation during sewage treatment they may release estrogenic alkylphenols. The surfactant nonoxynol is used as intravaginal spermicide and condom lubricant. When administered to lab animals it is metabolized to free nonylphenol. Bisphenol-A was found to contaminate the contents of canned foods; these tin cans are lined with lacquers such as polycarbonate. Bisphenol-A is also used in dental sealants and composites. We found that this estrogen leaches from the treated teeth into saliva; up to 950 μg of bisphenol-A were retrieved from saliva collected during the first hour after polymerization. Other xenoestrogens recently identified among chemicals used in large volumes are the plastizicers benzylbutylphthalate, dibutylphthalate, the antioxidant butylhydroxyanisole, the rubber additive *p*-phenylphenol and the disinfectant *o*-phenylphenol. These compounds act cumulatively. In fact, feminized male fish were found near sewage outlets in several rivers in the U.K.; a mixture of chemicals including alkyl phenols resulting from degradation of detergents during sewage treatment seemed to be the causal agent. Estrogen mimics are just a class of endocrine disruptors. Recent studies identified antiandrogenic activity in environmental chemicals such as vinclozolin, a fungicide, and DDE, and insecticide. Moreover, a single chemical may produce neurotoxic, estrogenic and antiandrogenic effects. It has been hypothesized that endocrine disruptors may play a role in the decrease in the quantity and quality of human semen during the last 50 y, as well as in the increased incidence of testicular cancer and cryptorchidism in males and breast cancer incidence in both females and males in the industrialized word. To explore this hypothesis it is necessary to identify putative causal agents by the systematic screening of environmental chemicals and chemicals present in human foods to assess their ability to disrupt the endocrine system. In addition, it will be necessary to develop methods to measure cumulative exposure to (a) estrogen mimics, (b) antiandrogens, and (c) other disruptors. © **1998 Elsevier Science Ltd. All rights reserved.**

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*Correspondence to C. Sonnenschein. Tel. 636 7451; Fax: 636 6536; e-mail: csonnenschein@infonet.tufts.edu.

INTRODUCTION

Barbara McClintock said [1]:

> We've been spoiling the environment just dreadfully and thinking we were fine, because we were using the techniques of science. Then it turns to technology, and it's slapping us back because we did not think it through. We were making assumptions we had no right to make... We're not thinking it through, just spewing it out...

For over half a century, substantial evidence has surfaced on the hormone-like effects of environmental chemicals in fish, wildlife and humans [2]. This activity was discovered long after these chemicals were released into the environment. The insecticide DDT was the first intentionally released chemical found to be estrogenic. In 1949, aviation crop dusters handling DDT were found to have reduced sperm counts [3]. Two decades later, workers at a plant producing the insecticide kepone were reported to have lost their libido, became impotent and had low sperm counts [4]. These cases of occupational exposure to pesticides provided evidence that these compounds may be estrogenic. Subsequently, experiments conducted in laboratory animals unambiguously demonstrated their estrogenic activity.

In addition to the occupational exposure cases, where a single agent is identified as the culprit, wildlife showing signs of reproductive damage are exposed to a combination of chemicals. Abnormal development of the reproductive system in gull embryos exposed *in ovo* to DDT and other pesticides suggested that these chemicals were the causal agents; experimental intoxication of eggs with DDT resulted in similar anomalies to those present in the specimens collected in their natural habitat [5]. Similarly, the decreased reproductive success of alligators and turtles in Lake Apopka, FL, could be linked to a spill of kelthane, a pesticide formulation containing DDE [6]. More often, decreased reproductive success, and signs of feminization and demasculinization in wildlife could not be ascribed to a single agent. Chemical analysis of the specimens revealed the presence of multiple compounds, some of them known to have hormone-like activity [2].

Man-made compounds were accidentally found to be estrogenic because they disrupted experiments conducted in laboratories studying the effects of natural estrogens. In our laboratory, an estrogen-like substance used in the manufacture of plastic centrifuge tubes was being released into biological material (plasma) stored there. We purified this activity and found it to be due to nonylphenol, which is used as an antioxidant in the manufacture of plastics [7]. Similarly, experiments conducted by Krishnan *et al.* while searching for an estrogen binding protein in yeast, demonstrated the presence of an estrogenic compound in their cultures that was identified as bisphenol-A [8]. This chemical was being released during the autoclaving of the polycarbonate plasticware used to sterilize the yeast culture medium.

The relevance of these accidentally discovered estrogens to wildlife and human health is now starting to emerge. Feminized male fish were found near sewage outlets in several rivers in the U.K.; a mixture of chemicals including alkyl phenols resulting from degradation of detergents during sewage treatment seemed to be the causal agent of this endocrine disruption [9]. Domestic sewage also produced feminization of male fish attributed to ethynyl estradiol, the synthetic estrogen used in oral contraceptives. Bisphenol-A was found to contaminate the contents of canned foods; these tin cans are lined with lacquers such as polycarbonate to prevent direct contact between the metal and the preserved food [10]. Hence, hormone-like activity of environmental contaminants is usually discovered accidentally, and toxicological tests used to regulate the release of chemicals into the environment, or their use in the food industry, do not assess their hormone-like activity. Moreover, the chemical structure of these compounds does not obviously resemble that of steroid hormones; hence, these estrogenic effects were totally unexpected.

During the last 3 y, some environmental chemicals were found to antagonize the effects of androgens; exposure of male fetuses *in utero* resulted in abnormal development of the genital tract [11]. These new data indicate that reproductive effects of environmental chemicals may be of a complex nature affecting several targets. That is, a single agent may have pleiotropic effects; for example, DDE is both an estrogen agonist and an androgen antagonist [12]. As we learn more about this subject, the complexity of the problem increases. In view of this trend, how are we to assess risk over a baseline of multiple exposure to chemicals already present in the environment, acting cumulatively and interacting through several pathways?

Explaining the effects of endocrine disruptors

The endocrine and reproductive effects of environmental contaminants are believed to be due to their (1) *mimicking* endogenous hormones such as estrogens and androgens, (2) *antagonizing* normal, endogenous hormones, (3) *altering* the pattern of synthesis and metabolism of natural hormones, and (4) *modifying* hormone receptor levels. It is relatively easy to conceptualize how chemicals that interfere with endocrine function via the pathways mentioned above affect adult individuals; most of these effects are reversible once the exposure ceases, as seen in cases of occupational exposure to estrogenic pesticides. In contrast, the consequences of developmental exposure during organogenesis may result in irreversible deleterious effects, as those observed by *in ovo* exposure to DDT in birds [2, 5] and *in utero* exposure

to DES in rodents and humans [13–16]. Finally, the list above is probably incomplete, since it is based on our rudimentary knowledge of hormone action via the classical receptor pathway; other pathways may also be involved.

Searching for a definition of estrogens

Natural estrogens are involved in the development and adult function of organs of the female genital tract, neuroendocrine tissues and the mammary gland; their role in reproduction spans from maintenance of the menstrual cycle to pregnancy and lactation. At the cellular level, they promote cell proliferation and hypertrophy of female secondary sex organs and induce the synthesis and secretion of cell type-specific proteins [17]. These effects are mediated through the estrogen receptors (ER). However, receptors are necessary but not sufficient to allow the recipient cell (in transfection experiments) to perform all the functions present in a natural target cell. Recently, a novel estrogen receptor was identified. The ''classical'' receptor, ERα, is present in the classical estrogen-target organs, while the newly recognized ERβ is present in the prostate, testis, ovary, and some areas of the brain [18, 19].

Exposure to natural estrogens is the principal risk factor in the development of breast and endometrial cancer. Exposure of adult human males to estrogens during adulthood results in gynecomastia and interferes with the normal function of the hypothalamus–hypophyseal-gonadal axis, resulting in decreased libido, impotence, decreased blood androgen levels, and lowered sperm counts. In utero exposure results in cryptorchidism, persistence of Mullerian remnants in males, enlarged prostates, vaginal adenosis, malformations of the female genital tract, and clear cell adenocarcinoma of the vagina [2, 20].

Humans and wildlife are exposed to non-gonadal estrogens such as phytoestrogens. The apparent lack of deleterious effects of phytoestrogens on human health may be explained by adaptive phenomena during co-evolution of plants and mammals; these adaptive phenomena are thought to develop slowly. Sudden exposure of organisms to phytoestrogens which are foreign to their natural ecosystems may result in deleterious health effects [21]. Xenobiotics of widely diverse chemical structure have estrogenic properties [22, 23]. This diversity makes it difficult to predict the estrogenicity of chemicals solely on structural bases. To overcome this shortcoming, their identification as estrogens has relied upon *bioassays*.

Methods for assessing estrogenicity

The foremost question in this field remains whether or not a given chemical is an estrogen. Diverse animal models and assays have been used to measure estrogenicity. Allen and Doisy and other pioneers of research on estrogens used mouse and rat activity units to follow their estrogen purification protocol; the end point of their assay was vaginal cornification [24]. The feminization of the feather pattern in brown leghorn capons [25] is another bioassay based on estrogen-mediated effects on gene expression. Rodent assays are indirect estimates of the proliferative effect of estrogens; they measure either vaginal cornification or the increase in uterine wet weight (uterotropic assay) [26]. The uterotropic assay is performed in a variety of versions: using single or multiple doses of estrogens over 24–72 h periods in immature or ovariectomized mice and rats. This diversity of end points indicates that there is no universal ''gold standard'' of estrogen action among animal bioassays. However, the *proliferative* effect of natural estrogens on the female genital tract has remained the hallmark of estrogen action. In fact, Hertz argued convincingly that this proliferative property should be adopted as the one that determines whether or not a chemical is an estrogen [17]. This requires measuring the increase of mitotic activity in tissues of the female genital tract after estrogen administration. However, this approach is not suitable for large-scale screening of suspected chemicals and an equally reliable, easy and rapid method would be preferable, such as that using established estrogen-sensitive cells in culture to measure the proliferative effect of xenoestrogens [27]. The E-SCREEN assay was developed to fulfil these requirements. Most of the recently discovered xenoestrogens have been identified with this method [28–30].

To obviate problems inherent to animal testing, quantitative bioassays using cells in culture have been developed to screen large number of chemicals for estrogenic activity in addition to the E-SCREEN assay. They are based on estrogen-induced gene expression, both of endogenous genes and reporter genes. For example, the induction of prolactin in primary sheep pituitary cell culture has been proposed as a measure of estrogen action [31]. In this model, estrogens induce protein synthesis but are ineffective at inducing cell proliferation. The limitations of these assays are that some estrogen-inducible genes could also be induced by non-estrogenic substances. For example, prolactin synthesis may be induced by epidermal growth factor, thyrotropin releasing factor and phorbol esters [32]. Another estrogen-inducible marker, ovalbumin synthesis, is stimulated by other steroids such as progesterone and glucocorticoids [33]. Estrogen-induction of endogenous, specific genes, such as progesterone receptor and pS2 in MCF7 cells, may also be used to screen for xenoestrogens [28, 34]. Induction of reporter genes under the control of estrogen-responsive elements have been proposed to assess estrogenicity; however, elevated basal expression in the absence of estrogen often occurs and this may raise concern about the reliability of these assays. Several yeast-based assays

were developed in which both estrogen receptor and a reporter gene are constitutively expressed. The cell walls and transport systems present in their cell membranes affect the intracellular levels of certain xenoestrogens. This results in artifactually low potency of these chemicals [35]. In addition, in some yeast models, the rate of false negatives is high [36, 37]. Moreover, like receptor binding assays, the yeast models do not differentiate agonists from antagonists. Recently, a stable transformant of MCF7 with an estrogen-inducible reporter gene was introduced [38]. This cell line is likely to be free of the drawbacks of the yeast models. However, this assay has yet to be tested regarding its usefulness to detect xenoestrogens. There is concern that reporter gene assays will result in false positives due to the phenomenon of "cross-talk" among structurally different ligands that bind to different receptors [39, 40]. Several publications compare the advantage and drawbacks of bioassays used to characterize suspected xenoestrogens [17, 22, 29, 30, 41, 42].

Development and proposed use of other bioassays using target cells in culture for the detection of endocrine disruptors

"In culture" screens. The MCF7 cell proliferation assay may also be used to assess the antiestrogenic properties of chemicals. For example, by simultaneously exposing cells to the minimal dose of estradiol required to induce maximal cell proliferation and to a range of concentrations of a putative antagonist, the assay detects both the magnitude of the antagonistic effect on the cell number, as well as the dose of antagonist necessary to cancel the proliferative effect of estradiol. To ascertain whether the effect on cell number is truly due to estradiol antagonism, a "rescue" experiment is performed, whereby the ability of a range of estradiol doses to reverse the antagonistic effect of the putative antiestrogen is tested. A chemical is considered a true antagonist when (a) it does not affect cell number in the absence of estrogens, (b) it blocks the proliferative effect of estradiol, and (c) this block is reversed by merely increasing the dose of estradiol while keeping constant the dose of the antagonist. Using this assay, dioxin was found to decrease the number of MCF7 cells in the absence of estrogens, and to block the proliferative effect of estradiol, but this effect was not reversed by increasing doses of estradiol. This implies that the effect of dioxin may be one of general toxicity rather than a truly specific antiestrogenic effect [43].

We developed the androgen proliferative screen assay (A-SCREEN) using the human prostate carcinoma LNCaP-FGC cells [44, 45] which exhibit a biphasic proliferation response to androgens. At low androgen doses, these cells increase their proliferation rates, while at higher doses, androgens inhibit their proliferation. Most recently, we selected an LNCaP-FGC variant, the LNCaP-TAC cell line [46] that can be used advantageously to perform the A-SCREEN assay. LNCaP-TAC cells are inhibited from proliferating when exposed to medium supplemented with serum treated with charcoal-dextran to remove androgens. Androgens induce a monophasic proliferative response comparable to that evoked by estrogens in MCF7 cells. To date, when using either animal models or androgen-sensitive cells, no androgen agonists have been found among environmental chemicals. However, using the LNCaP-TAC cell proliferation assay to detect antagonists, we verified the finding previously reported by Gray *et al.* using a rodent model, namely that DDE is an androgen antagonist [12]. However, the androgen receptor in LNCaP cells was reported to have a point mutation in its androgen-binding domain; this increases its affinity for estrogens. To circumvent this problem, we developed a stable androgen receptor transfectant of MCF7 cells (MCF7-AR1) [47]. This cell line specifically responds to androgens by decreasing its proliferation rate (proliferative shutoff).

We propose the use of a battery of screening assays such as the one described above to be used during the development of chemicals expected to be released into the environment, as well as chemicals destined to come in contact with food. Since these assays are inexpensive and easy to perform, they may be run simultaneously and rapidly to obtain a "sex hormone activity profile" of the chemical. In addition, to the screening for estrogen and androgen agonists and antagonists, it would be desirable to test chemicals for other endocrine disrupting properties, such as those affecting the thyroid and adrenal glands. In the end, when the chemical is ready to undergo final testing in animals to assess developmental and transgenerational toxicity, the likelihood of finding undesirable effects would have been greatly reduced because of early weeding out of compounds producing unacceptable endocrine-disrupting effects [29].

Markers of exposure. Humans and wildlife are *simultaneously* exposed to a variety of chemicals [48, 49]. Residues of diverse estrogenic xenobiotics coexist in fat and body fluids of exposed individuals [49]; hence, it is likely that they may become bioavailable. At such time, they may act cumulatively, i.e., when each of them is present at levels lower than those needed to express overt estrogenicity. We explored this concept and found that xenoestrogens act cumulatively to induce cell proliferation when administered to MCF7 cell cultures. Hence, measuring the total estrogenic burden due to environmental contaminants present in plasma/tissue samples may be more meaningful than assessing exposure by measuring the levels of each of the known xenoestrogens singly. These considerations have implications for the study of human conditions that are suspected to be caused by environmental estrogens, such as undescended testis (cryptorchidism),

breast cancer and the decrease of sperm counts and quality during the last 50 y [50–52]. A bioassay such as the MCF7 cell assay may be used to measure the xenoestrogen burden of organisms once a protocol is developed to separate environmental estrogens from endogenous ones, a process that is now being developed in our labs [30, 53].

Recently identified estrogens

Novel xenoestrogens were found among antioxidants, plasticizers, detergents, PCB congeners, and pesticides.

(1) Alkylphenols are used as antioxidants and in the synthesis of detergents [alkylphenol polyethoxylates, (APEs)]. APEs are used as industrial detergents in the textile and paper industries, in toiletries, and as spermicides. Four hundred and fifty million pounds of APEs were sold in the U.S. in 1990 [54]. APEs are not estrogenic per se; however, they are degraded during sewage treatment. The polyethoxylate chain is shortened, and free alkylphenols as well as mono and diethoxylates are produced. The free phenols are estrogenic [7, 27]. Recently, White *et al.* have shown that alkyl phenol diethoxylates are also estrogenic [55]. These APE degradation products have been detected in drinking water [56]. Nonylphenol has been reported to leach from PVC tubing for milk processing [57] and plastics used in food packaging [58]. APEs such as those used as spermicides are degraded to free nonylphenol when administered to rodents [59]. The contribution of APEs and alkyl phenols to the xenoestrogen burden of humans is unknown. It has been reported that these chemicals are present in certain sewage outlets in concentrations sufficient to feminize sentinel fish [9]. Alkylphenols accumulate in river sediment and in the fat of exposed fish [60]. Interesting observations pertaining to structure-function relationships were made: (i) the alkyl chain must at least have 3 carbons, (ii) only the *p*-isomers are estrogenic, (iii) polyalkylated, hindered phenols like butylated hydroxytoluene and Irganox 1640 (Ciba–Geigy) are not estrogenic while being effective antioxidants, and (iv) fused rings like naphthols are not estrogenic in spite of being an integral part of the A and B ring of natural steroids. Instead, substituted naphthols such as 6-Br naphthol and allenolic acid are estrogenic; more studies are needed to assess whether these substituted naphthols are active due to a "bulk" effect, electronegativity, or because flat molecules such as naphthols and coplanar PCBs are unable to bind tightly to the estrogen receptor [27, 28].

(2) Phenolic antioxidants such as alkylphenols are used in the manufacture of plastics and to protect petroleum against oxidative degumming [61]. Some phenolic antioxidants such as butylated hydroxytoluene (BHT) and butyl hydroxyanisole (BHA) are also used to prolong the shelf life of foodstuffs and to reduce nutritional losses by retarding oxidation. In addition to the estrogenic alkylphenol antioxidants described above, we found that BHA is estrogenic [28]. BHA is a widely used antioxidant and it is most effective to control oxidation of short chain fatty acids such as coconut and palm oils [62]. Maximal usage levels of BHA permitted by FDA vary according to the food type, from 50 ppm in dry breakfast cereals to 1,000 ppm in active yeast.

(3) Polychlorinated biphenyls (PCBs) have been accidentally released into the environment; they are used in transformers and other electrical equipment. Due to their chemical structure resembling that of DDT, some of these compounds were suspected to be estrogenic [63]. Korach *et al.*, demonstrated the ability of certain hydroxy-PCBs to bind to estrogen receptors and to produce an uterotropic effect that correlates with their relative binding affinity to the estrogen receptor [64]. It is generally assumed that hydroxylated PCBs are estrogenic while non-hydroxylated PCBs are not; however, 5 of the 18 congeners studied were estrogenic when assayed by measuring the proliferation of MCF7 cell cultures (the E-SCREEN assay) (Table 1). It is unknown whether these congeners were estrogenic per se or they were hydroxylated by MCF7 cells. Among the hydroxylated PCBs, those *para*-hydroxylated were more potent than *meta*-hydroxylated; *ortho*-hydroxylated compounds were even less active. As mentioned above while discussing alkylphenols, hindered phenols, such as in 4-hydroxy 3,5, DCB were less estrogenic than unobstructed ones [28].

(4) The pesticides dieldrin and toxaphene are estrogenic [65]; their use has been restricted in the U.S. since 1974 and 1982, respectively [65]. These compounds are highly lipophilic and bioaccumulate through ecosystems; they are still found in wildlife, coincidentally with signs of reproductive impairment. Toxaphene is a major air-borne pollutant in North America; its residues appeared in regions where it has never been used, like the Arctic and Scandinavia [66]. Arctic and Baltic salmon muscle fat has been shown to contain concentrations between 700 to 7,000 ppb [67]; this concentration is well within those producing estrogenic effects in the E-SCREEN assay (10 μM = 4,800 ppb). Endosulfan was introduced in 1954; it is presently used for agricultural purposes in the U.S. and other countries [68]. Proliferative, estrogen-like effects in MCF7 cells were found at doses of 10 μM (4,060 ppb). Endosulfan was shown to produce testicular atrophy in male rats fed a diet containing 10 ppm [69, 70]; it also lowered gonadotrophin and testosterone plasma levels [71]. These results may be explained by the recently reported estrogen mimicking properties of endosulfan [65].

Phenylphenol is one of the top ten active ingredients in household pesticides and is the second most

Table 1. *Xenobiotics tested by the E-SCREEN assay*

Estrogenicxenobiotics	Non-estrogenic xenobiotics	
I. Herbicides		
None	2,4 D	2,4 DB
	Alachlor	Atrazine
	Butylate	Cyanazine
	Dacthal	Dinoseb
	Hexazinone	Metolachlor
	Propazine	Picloram
	Trifluralin	Simazine
II. Insecticides		
pp′ DDT	Bendiocarb	Carbofuran
op′ DDT	Chlordane	Chlordimeform
op′ DDE	Diazinon	Chlorpyrifos
op′ DDT	Heptachlor	Carbaryl
DDT[a]	Kelthane	Lindane
Dieldrin	Malathion	Mirex
Chlordecone (kepone)	Methoprene	Pyrethrum
Endosulfan[a]	Parathion	
Alpha endosulfan	Rotenone	
Beta endosulfan		
Methoxychlor		
Toxaphene		
III. FUNGICIDES		
None	Chlorothalonil	Thiram
	Hexachlorobenzene	Zineb
	Maneb	Ziram
	Metiram	
IV. Industrial chemicals		
2,3,4 TCB	Butylated Hydroxytoluene	2 CB
2,2′,4,5 TCB	2,5 DCB	4 CB
2,3,4,5 TCB	2,6 DCB	2,3,6 TCB
2,4,4′,6 TCB	3,5 DCB	2,3,5,6 TCB
2,2′,3,3′,6,6′ HCB	2,3′,5 TCB	2,3,3′4,5′ PCB
2 OH-2′,5′ DCB	2,3,4,4′ TCB	2,2′,3,3′,5,5′ HCB
3 OH-2′,5′ DCB	2,3,4,5,6 PCB	2 OH-3,5 DCB
	2 OH-2′,3′,4′,5,5′ PCB	DecaCB
4 OH-2′,5′ DCB		
4 OH-2,2′,5 TCB	3 OH-3,5 DCB	Diamyl phthalate
4 OH-2′,4′,6′ TCB	4 OH-3,5 DCB	Dibutyl phthalate
3 OH-2′,3′,4′,5′ TCB	1,2-dichloropropane	Dimethyl isophthalate
	Irganox 1640	Dimethyl Terephthalate
4 OH-2′,3′,4′,5′ TCB		
4 OH-alkyl-phenols	2,3,7,8 TCDD	Dinonyl phthalate
Bisphenol-A	Tetrachloroethylene	Octachlorostyrene
4 OH-biphenyl		styrene
3 OH-biphenyl		
2 OH-biphenyl		
t-Butylhydroxyanisole		
Benzylbutylphthalate		

[a]Denotes a technical grade isomer mixture.

widely used ingredient for indoor applications [72]. We reported previously that *p*-phenylphenol is a full estrogen agonist [49]; *o*- and *m*-phenylphenol are partial agonists (Table 1). *o*-Phenylphenol is used in the rubber industry, in agricultural fungicides and in disinfectants; *p*-phenylphenol is used in the rubber industry and in the manufacture of resins. Among the three isomers of phenylphenol, *p*-phenylphenol was a full agonist and the most potent, *o*-phenylphenol was the least potent and a partial agonist. The *meta* isomer is also a partial agonist; its potency fell within the ranges of the other two [30].

(5) Bisphenol-A is used in the synthesis of polycarbonate plastics. It may leach from polycarbonate plastics due to incomplete polymerization or to breakdown of the polymer upon heating, as during sterilization by autoclaving [8]. Since this polymer is used in food and beverage packing, the potential to affect humans by ingestion of contaminated food and beverages is obvious. Olea *et al.* have reported the presence of bisphenol A in foods preserved by canning in polycarbonate-lined tin cans [10]. Equally significant, these researchers also found that bisphenol-A and bisphenol-A dimethacrylate, which is also estrogenic, leach from sealants applied to teeth, into saliva. Bisphenol-A based resins are also used in composites (white fillings) [34].

(6) Plasticizers are used to decrease the rigidity of certain polymers. For the most part, they are di- and triesters of organic acids. Phthalate esters are widely used plasticizers. These compounds slowly leach from plastics and they have been found to be ubiquitously distributed in the environment, including marine ecosystems [73]. Among the plasticizers in Table 1, a pattern is emerging whereby those containing aromatic residues are likely to be estrogenic, such as benzylbutylphthalate and diphenylphthalate. Benzylbutylphthalate is a widely used plasticizer in the manufacture of flooring tiles; it is also used as a plasticizer for cellulose plastics, polyvinyl acetates, polyurethanes, polysulfides, and regenerated cellulose films for packaging. It is also used in synthetic leathers, acrylic caulking, adhesive for medical devices and in the cosmetic industry. Other uses include that as a carrier and as a dispersant for insecticides, insecticide repellents and perfumes. Most interestingly, BBP is approved for use as a component of paper and paperboard in contact with liquid, fatty and dry foods [74].

All the newly identified estrogens tested so far not only induce cell proliferation but also increase the expression of pS2 and progesterone receptor (PgR) in MCF7 cells in culture. These xenoestrogens compete with estradiol for binding to its receptor; their relative binding affinities to the estrogen receptor correlated well with their potency to induce cell proliferation, pS2 and PgR [28].

CONCLUSIONS

This review provides a synopsis of the knowledge acquired so far by the use of "in culture" assays in identifying chemicals suspected of being estrogenic. This screening effort, by far incomplete, revealed that there are (1) estrogens among chemicals that by virtue of being in direct contact with food may pose a health risk for humans. So far, bisphenol-A, plastici-

zers such as benzylbutylphthalate and antioxidants such as BHA and alkyl phenols have been identified; (2) other sources of exposure are alkyl phenol polyethoxylates and benzylbutylphthalate used as dispersant agents for pesticides, and in cosmetics and toiletries. A general strategy is proposed to prevent the introduction of xenoestrogens and other endocrine disruptors through a systematic series of inexpensive and reliable "in culture" assays. Finally, we introduce the concept of measuring the total xenoestrogen burden using the MCF7 estrogenicity assay as a marker of exposure to environmental estrogens; this parameter may be used to assess the role of xenoestrogens on the increased incidence of breast cancer, cryptorchidism and the lowering of the quality of semen in human populations.

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