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Environmental oestrogens – present understanding

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Three years ago it was hypothesized that the reported adverse changes in male reproductive health could be explained by exposure to compounds with oestrogenic (or other hormone disruptive) activity. Although this issue has been highly publicized, there has been little progress towards a realistic assessment of whether environmental oestrogens pose a health risk to humans. This article attempts to give a brief overview of the current status of knowledge concerning environmental oestrogens and highlights some of the difficulties associated with risk assessment. Compounds within several major groups of chemicals, including organochlorine pesticides, polychlorinated biphenyls, phenolic compounds and phthalate esters, have been identified as being weakly oestrogenic by *in vitro* and *in vivo* screening methods. Many of these compounds are widespread and persistent in the environment. They are likely to be present in the food chain, drinking water, plastics, household products and food packaging, although which is the most important route of human exposure is unclear. In addition to exposure to man-made chemicals, the consumption of plant-derived oestrogens in foodstuffs poses a potential risk to human health as phytoestrogens are more potent oestrogens and their intake by some infants is likely to be considerable.

From what has been printed in some newspapers the reader would most likely conclude that 'environmental oestrogens' are a proven risk to human health. In reality, there is no such proof and it is equally possible that such compounds pose no significant risk to humans. Distinguishing between these two extremes is very important, as testified by the number of meetings, government reports and research initiatives in the past 3 years. There is general agreement that the original hypothesis suggesting that human exposure to compounds with oestrogenic (or other hormone-disruptive) activity could be responsible for the reported adverse changes in male reproductive health (increase in testicular cancer, decline in sperm counts, increase in testicular/genital malformations; see Sharpe and Skakkebaek, 1993) is biologically plausible. However, relatively little progress has been made towards confirming or refuting this hypothesis over the past 3 years. One reason for this lack of progress has been the absence of known biological endpoints of oestrogen action in males and uncertainties as to which are the most important oestrogenic chemicals to be investigated. In addition, a number of recent discoveries have complicated the task of assessing when and how environmental oestrogens might affect the body. Chief among these have been (1) the discovery of a second oestrogen receptor (ERβ; Kuiper *et al.*, 1996); (2) the demonstration that oestrogenic chemicals/oestrogen can potentially induce biological effects via non-conventional pathways (Yang *et al.*, 1996), leading to the suggestion that the biological effects of particular oestrogens may be tissue-specific; and (3) the demonstration *in vitro* that weakly agonistic oestrogenic chemicals can have additive effects even when in the presence of a potent agonist such as oestradiol (Jobling *et al.*, 1995; Sumpter and Jobling, 1995). Synergistic effects between oestrogenic pesticides have also been reported (Arnold *et al.*, 1996a); however, this remains a contentious issue as other studies have been unable to reproduce these results (Ashby *et al.*, 1997; Ramamoorthy *et al.*, 1997).

It is also remarkable that, in Europe at least, hardly any attention has been paid to the potential adverse effects that environmental oestrogens may exert in females considering that (1) endpoints to make such an assessment are probably more straightforward than in males, and (2) oestrogen-modulated diseases, such as breast cancer, are of such importance in humans.

Environmental oestrogens, plus other potential hormone-disrupting chemicals, has grown into an enormous topic in the past few years and, in the space of this short review, it is only possible to address certain aspects. We have chosen, therefore, to concentrate on discussing briefly the sorts of chemicals that have been identified as being oestrogenic, considering the potential routes of human exposure (compared with animals) to these chemicals, and identifying important issues in this area that need to be addressed. For more details on other aspects of environmental oestrogens the reader is referred to the most recent in-depth report (Toppari *et al.*, 1996).

Environmental man-made chemicals with known oestrogenic effects

The man-made chemicals with known oestrogenic effects identified so far can be divided into several major groups of chemicals such as organochlorine pesticides, polychlorinated biphenyls (PCBs), phenolic compounds and phthalate esters. It should be emphasized that only some of the chemicals within each group have been shown to be oestrogenic and only a small fraction of man-made chemicals present in our environment have been tested. It is reasonably certain that other chemicals with oestrogenic activity have yet to be identified. The process of predicting whether a chemical is oestrogenic is hindered by

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Table 1. Environmental chemicals with known oestrogenic effects *in vitro*

Chemical	Reproductive effects	Human exposure	Potential routes of exposure
Organochlorine pesticides			
DDT, methoxychlor, dieldrin, kepone	Well-documented for wildlife and laboratory animals	High, especially in the 1940s–1960s	Ubiquitous in the environment; food contamination
Industrial chemicals			
Polychlorinated biphenyls: 3,4,3',4'-tetrachlorobiphenyl	Well-documented for wildlife and laboratory animals	High, especially in the 1940s–1960s	Ubiquitous in the environment; food contamination
Alkylphenolic compounds: nonylphenol, octylphenol	Poorly documented; adverse effects in aquatic species	Unknown	Surfactants; used in most plastics; also present in drinking water, household products, food packaging, some cosmetics, shampoos, spermicides
Phthalate esters: butylbenzyl phthalate, di-*n*-butyl phthalate	Reasonably well-documented effects on laboratory animals at high doses	High	Used in plastics, PVC and many other products; present in food and food packaging, drinking water, household products
Bisphenol-A and its derivatives	No published data	Unknown	Used in polycarbonate plastics, acrylic resins, xeroxing, dentures and in the lacquer coating of food cans; some food packaging
Food additives			
Butylated hydroxyanisole	No obvious effects from toxicity data	Moderate	Food antioxidant
Phytoestrogens			
Isoflavones: genistein, daidzen Coumestans: coumesterol	Well documented effects in animals; some evidence in humans	High (very high on a soy-rich diet)	Many natural foods, especially soy products; many processed foods (soy); soy-formula infant milk

the wide difference in structure of the oestrogenic chemicals so far identified, although increasingly it appears that phenolic compounds are likely to be oestrogenic. Some examples of environmental oestrogens are listed (Table 1) and are discussed briefly below (for more detailed information see Toppari *et al.*, 1996).

Several of the oestrogenic chemicals identified are organochlorine pesticides, for example, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT), methoxychlor, kepone (chlordecone) and dieldrin. DDT was used in large quantities until the 1960s when its use was banned or restricted in developed countries but it is still used widely in developing countries. DDT has a half-life of > 60 years and bioaccumulates in body fat. Several isomers of DDT exist but *o,p'*-DDT is the most oestrogenic, as demonstrated by its oestrogen receptor binding (Kelce *et al.*, 1995) and its uterotrophic activity *in vivo* (Gellert *et al.*, 1972). However, the principal long-lived metabolite of DDT in the body is *p,p'*-DDE which can act as a potent anti-androgen *in vitro* and *in vivo*. It is now reckoned that the anti-androgenic effects of *p,p'*-DDE may be more important toxicologically than the oestrogenic effects of *o,p'*-DDT (Kelce *et al.*, 1995; Kelce and Wilson, in press). For example, *p,p'*-DDE has been implicated in reproductive abnormalities observed in Lake Apopka alligators (Guillette *et al.*, 1996). Both methoxychlor and chlordecone (kepone) are oestrogenic *in vitro* and *in vivo*, and neonatal administration of either to female rats results in accelerated vaginal opening and early onset of persistent oestrus (and therefore infertility) in adulthood (Gray, 1992); the latter is analogous to polycystic ovarian disease in women (Franks, 1993), the cause of which remains unclear. Other pesticides such as dieldrin, toxaphene and endosulfan are oestrogenic *in vitro* (Soto *et al.*, 1994).

Polychlorinated biphenyls (PCBs) are widespread and persistent in the environment. They comprise a mixture of 200 or more congeners, some of which are oestrogenic *in vivo* and *in vitro* while others may be anti-oestrogenic (Jansen *et al.*, 1993; Krishnan and Safe, 1993). Recently, 3,4,3',4'-tetrachlorobiphenyl, a PCB congener known to be abundant in human tissues, was shown to bind to the oestrogen receptor, to induce proliferation of human breast cancer cells and to increase uterine weight *in vivo* (Netsaretnam *et al.*, 1996).

Alkylphenols and alkylphenol polyethoxylates (APEOs) are used widely as industrial surfactants in detergents, paints, herbicides, pesticides and cosmetics, as antioxidants in most plastics, as petrol additives and as spermicides in condoms. Millions of kilograms are produced annually of which 60% can end up in the aquatic environment. Alkylphenols, such as nonylphenol and octylphenol, are formed by the microbial breakdown of APEOs during sewage treatment and are relatively persistent in the environment. Several alkylphenolic compounds (octylphenol, nonylphenol, nonylphenoxycarboxylic acid and nonylphenoldiethoxylate) are unequivocally oestrogenic *in vitro* (White *et al.*, 1994) and *in vivo* (Jobling *et al.*, 1996).

Bisphenol-A and its derivatives are again used widely: in polycarbonate plastics, in acrylic resins, in false teeth, in xeroxing, in certain fungicides and in the lacquer coating of food

cans. They may leach out of all of these products and are clearly oestrogenic *in vitro* (Krishnan *et al.*, 1993; Brotons *et al.*, 1995) and *in vivo* (Bitman and Cecil, 1970).

Phthalates, which are used as plasticizers, are among the most ubiquitous man-made chemicals present in the environment and human intake is in milligrams per day. Some phthalate esters (for example, butyl benzyl phthalate and di-*n*-butyl phthalate) are oestrogenic *in vitro* though it appears that most are non-oestrogenic. Finally, a widely used food antioxidant, butylated hydroxyanisole, has also been shown to be weakly oestrogenic *in vitro* (Jobling *et al.*, 1995). It is highly likely that other chemicals, especially some phenolic/biphenolic compounds (or their metabolites), which comprise > 50% of all man-made chemicals, will prove to be oestrogenic when tested.

Although the chemicals described above have been shown to be oestrogenic, in every instance they are weak oestrogens, that is, 10^3–10^4-fold less potent than oestradiol, although this measure of potency takes no account of bioaccumulation or whether the chemical binds to steroid-binding proteins in plasma (Arnold *et al.*, 1996b). Nevertheless, it is reasonable to assume that biological effects of individual compounds in animals or man will occur only when exposure is reasonably high. For this reason, it has been argued that exposure to such chemicals is of minor significance compared with exposure to naturally derived plant oestrogens.

Phytoestrogens

Phytoestrogens are present in moderate to substantial amounts in plants (soya, beans, grains, vegetables and fruit) and are consumed by both man and animals. They are structurally and functionally similar to oestradiol and there are three main types: isoflavones (for example, genistein, daidzein), coumestans/lignans (for example, coumesterol) and mycoestrogens (for example, zearanalone) (Kaldas and Hughes, 1989). Unlike many of the chemicals described above, phytoestrogens do not bioaccumulate in body fat and are readily metabolized. However, in contrast to the chemicals discussed above, phytoestrogens are considerably more potent oestrogenically and intake per day is substantial.

Environmental oestrogens and risk to reproductive health

Oestrogenic chemicals

If sufficient exposure to an oestrogenic chemical occurred, it would presumably cause biological effects, which might be harmful. There is no direct evidence to show such a cause and effect relationship in humans and although there are some examples for wildlife (fish, alligators, birds, domestic animals; see Toppari *et al.*, 1996), in only one of these instances is there a strong link with exposure to a particular man-made chemical (alligators; Guillette *et al.*, 1996) and then it was to *p,p'*-DDE, acting not as an oestrogen but as an anti-androgen. Most environmental oestrogenic chemicals have been identified based on one or more *in vitro* screening systems (human breast cancer cells, fish hepatocytes or transfected yeast cells) and there is relatively little data on their bioactivity *in vivo*, in particular whether they are able to exert effects at environmentally relevant concentrations (Toppari *et al.*, 1996). Some information is beginning to appear for laboratory animals and for fish in test situations but insufficient to allow for definitive conclusions to be drawn. Without good data on effects *in vivo* of the suspect chemicals in animals and parallel information on the extent of human exposure (for which the data on non-pesticide chemicals are very poor), it is difficult to evaluate the risk to humans (Fig. 1). Other obstacles to risk assessement are (1) *in vitro* data suggesting additive effects of oestrogenic chemicals which are extremely difficult to study *in vivo* (Soto *et al.*, 1994; Jobling *et al.*, 1995; Sumpter and Jobling, 1995), and (2) in males at least, the absence of definitive biological endpoints of oestrogen exposure. Recent indications that oestrogens may be important for functionally normal reproductive development in males (Eddy *et al.*, 1996) suggest that such endpoints are identifiable.

The general assumption for animals is that exposure to environmental oestrogens is via food and water and this seems reasonable. Whether the same is true for humans is less certain. In Western societies, contamination of drinking water is likely to be of minor significance, although food and food packaging may be important sources of some oestrogenic chemicals. There may be other more important (and unique) routes of human exposure to such chemicals from substances such as the many lotions, creams, shampoos, antiperspirants that we now apply to our skins daily. Although there is no published evidence to show that these products are oestrogenic, they are likely to contain one or more of the chemical types described above. Therefore, they should be an important target for future research.

Phytoestrogens

There is strong, athough indirect, evidence that consumption of a high phytoestrogen-containing diet (like that prevalent in Japan) may give some protection against breast cancer and, as a result, phytoestrogens have tended to be viewed as being beneficial rather than harmful to man (Messina *et al.*, 1994). This is despite unequivocal evidence from a number of animal species that phytoestrogen consumption can interfere with reproductive development and function (Kaldas and Hughes, 1989), and recent evidence in humans that consumption of a high soy-containing diet can prolong the follicular phase of the menstrual cycle by suppressing FSH and LH secretion (Cassidy *et al.*, 1994). Because it is cheap, soy-derived protein has been used increasingly in processed foods in the West over the past 20 years or so, and it is reckoned that 60% of such foods now contain soy derivatives. Little consideration has been given to the possibility that this could be harmful or that at least it may be altering our overall exposure to 'oestrogens', probably because it is considered that it does not appear to harm oriental societies who have consumed such a diet for centuries. This thinking makes all manner of presumptions, especially when extended to baby-foods. Contrary to what has been presumed, recent evidence shows that babies who are fed a 100% soy-formula milk diet (about 10% of babies in UK) have blood concentrations of isoflavenoids approximately 1000 times higher than in infants who are being breast-fed by mothers on a soy-rich (oriental) diet (K. D. R. Setchell, presented at 2nd International Symposium on Soy Foods and the Prevention of Chronic Disease, Brussels, 1996). In view of the evidence that exposure to phytoestrogens in the lactational period can result in late onset adverse effects

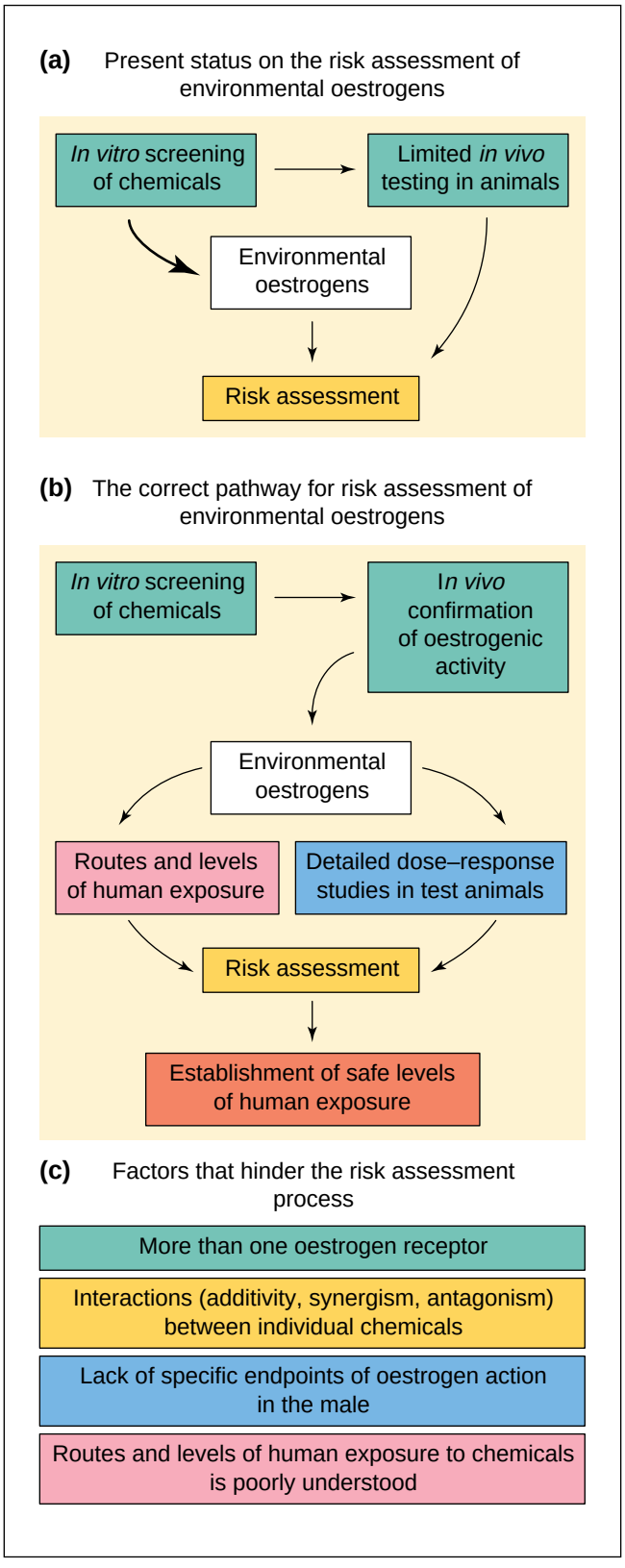

Fig. 1. Contrast between (a) present understanding of the risk that environmental oestrogens pose to human health and (b) the complete risk assessment process with (c) some of the factors that complicate this process.

Box 1. Current understanding of the effects of environmental oestrogens on humans

The list of environmental oestrogens is incomplete. It is therefore unknown whether the most important oestrogenic chemicals have been identified.

Emphasis has been on *in vitro* screening of chemicals for oestrogenicity. This may not equate with oestrogenic activity *in vivo*.

Addition of soy-derived protein (and thus phytoestrogens) to food on a widespread scale poses potential risks to human health, particularly to some infants.

Recent evidence shows that oestrogens are likely to be important for normal reproductive function in males. The biological actions of oestrogen in the male are poorly understood.

A certain level of exogenous exposure to oestrogen could be beneficial to the health of some groups of people.

in female rodents (rodent equivalent of polycystic ovarian disease) in adult life (Clarkson *et al.*, 1995), it is clearly urgent to check for such effects in Western societies.

Concluding remarks

An often quoted phrase is that we now live in a sea of oestrogens, and there is clearly some truth in this. However, it is another matter entirely to conclude that this must be bad for us, unless we have the evidence to support this supposition. Hopefully, this short review has illustrated that there are many unknowns standing between us and a realistic assessment of whether environmental oestrogens pose any risk to humans (Fig. 1) or, indeed, whether they might be beneficial to some (for example, postmenopausal women) or even most individuals. For those of us involved in research in reproduction, the notion that we can unwittingly be exposed to a number of 'oestrogens', no matter how impotent they may be, understandably fills us with concern. It must therefore be a priority to fill the gaps in our understanding, for no matter what the final conclusion may be regarding risk to humans, we cannot fail to be better off being well-informed than being ignorant. It is remarkable that it is nearly 60 years since the first report was published (Dodds and Lawson, 1938) showing that a surprising number of man-made organic chemicals were oestrogenic *in vivo*, yet until a few years ago most of us were ignorant of this. In the interim, all manner of changes in childhood growth, age at puberty and incidence of hormone-modulable diseases/disorders (breast and prostate cancer, polycystic ovarian disease) have occurred in humans. While there may be rational explanations for these changes that have little or nothing to do with exposure to hormones, when we know that 'oestrogens' can arguably affect all of these parameters it is surely foolhardy to dismiss the possible involvement of environmental oestrogens just because we have no data. As toxicologists are fond of saying 'absence of evidence is not evidence of absence'.

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