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Environmental oestrogens and human reproductive cancers

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Introduction

The past 4 years have seen an explosion of interest and concern with respect to 'environmental hormone disruptors', particularly environmental oestrogens. This is a consequence of (i) the discovery that a range of widely used man-made chemicals (including certain pesticides, alkyphenols, phthalate esters and phenolic compounds), to which we are exposed daily, can act as weak oestrogens, and (ii) their hypothetical links to an increase in disorders of reproductive development and function in the human male and in some species of wildlife (Toppari *et al.* 1996). Additionally, several other ubiquitous chemicals to which man has had considerable exposure over the past half-century are potent anti-androgens (notably the fungicide vinclozolin (Kelce *et al.* 1994) and the principal, and most persistent, metabolite of *p,p′*-dichlorodiphenyltrichloroethane (DDT) in the body, *p,p′*-1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) (Kelce *et al.* 1995)). These discoveries have prompted numerous television programmes, government-funded enquiries and reports and have even led to legislative changes in the USA. Inevitably, speculation and exaggerated claims have appeared in the popular press, but these have generally not been based on solid scientific foundation (see Kavlock *et al.* 1996, Ashby *et al.* 1997). On the other hand, the coincidence is striking between human exposure to these man-made chemicals and the apparent increase in prevalence of a range of hormone-dependent human cancers of reproductive tissues (breast, prostate, endometrium, testis and ovary). We therefore need to know whether these chemicals are directly involved in the aetiology of any of these cancers, so that if necessary human exposure to such chemicals can be reduced. Unfortunately, as this review will demonstrate, obtaining the data which would enable unequivocal, informed risk assessment is anything but straightforward (Kavlock *et al.* 1996, Ashby *et al.* 1997).

Whilst our present level of knowledge provides little direct evidence linking human exposure to 'hormone-disrupting' chemicals and the subsequent occurrence of cancer or any other disease/disorder of the reproductive system (Kavlock *et al.* 1996, Ashby *et al.* 1997), it is equally impossible to say with certainty that such links do not exist. There is simply an absence of definitive data. This may seem rather puzzling to the reader who is unfamiliar with this area but it becomes understandable when it is realised that answers are not available to fundamental questions such as: is there a critical time of exposure to hormones or hormone-disrupting chemicals - if so is it in adulthood, infancy, neonatally, or *in utero*, or is it a summation of exposure in multiple time-frames? The monitoring of exposure to an individual chemical during these time-windows in relation to development of cancer(s) which may appear many years later poses gargantuan technical and feasibility problems. If then one has to consider the sum of exposure to all of the relevant chemicals, the task becomes Herculean. There are also numerous other 'complications', which will be referred to below. The purpose in raising these problems up-front, is not to excuse lack of definitive conclusions but rather to put the reader's feet firmly on the ground at the outset. However, hormones are involved in the aetiology of many cancers of the reproductive system and the data for the incidence of hormone-dependent cancers as shown in Fig. 1 highlight the large scale of the problem. If many ubiquitous environmental chemicals can act as weak hormone agonists or antagonists, we cannot ignore their potential involvement in human disease just because the task of proving or disproving their involvement is complex and onerous. Hopefully, this review will contribute the first few steps in climbing this particular mountain.

A brief overview will first be provided of the evidence linking human exposure to endogenous or exogenous hormones (mainly oestrogens) to risk of cancers of reproductive organs. This is not intended to be definitive, and the reader seeking a more detailed analysis of this aspect should consult the papers which have been cited. The major proportion of this review is directed towards a detailed analysis of the various groups of chemicals which

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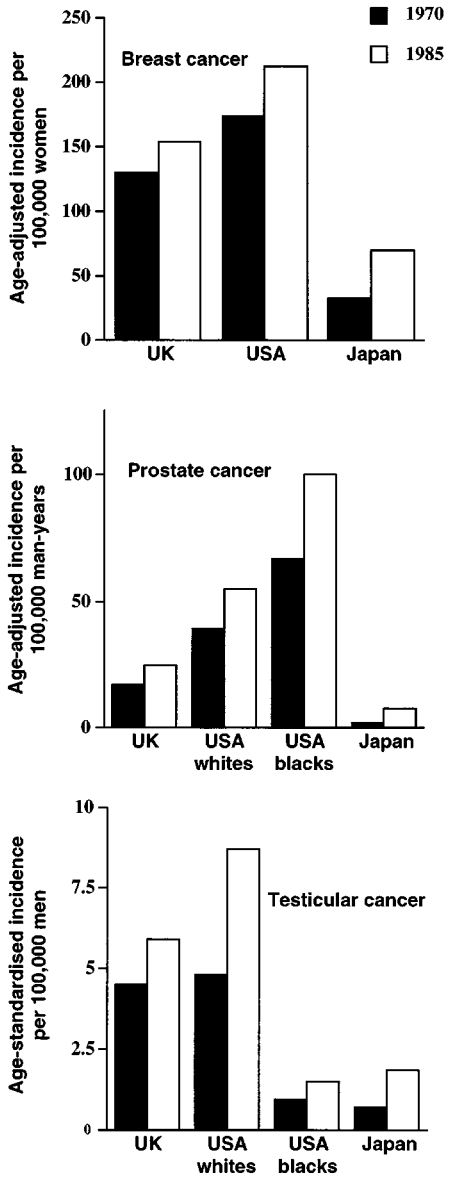

Figure 1 Changing incidence of cancers of the breast, prostate and testis in the period from 1970-1985 in the UK, USA and Japan. Note the considerable differences in cancer incidence between the USA/UK and Japan, but that despite this difference cancer incidence is increasing in all these countries. The difference in cancer incidence between USA/UK and Japan is thought to reflect dietary differences which may affect hormone exposure of the target tissues, and these are discussed in the text. However, ethnic differences can also be important as typified by the radically different incidence of cancers of the prostate and testis between black and white men in the USA, which may also reflect ethnic differences in hormone exposure in fetal and/or postnatal life. Adapted from data published in *Cancer Surveys: Trends in Cancer Incidence and Mortality* Volume 19/20 (1994); Imperial Cancer Research Fund, London.

have been identified as possessing hormonal activity (environmental hormone disruptors). Information will be presented on the uses and potential routes (and where known) levels of human exposure. Consideration will then be made of the epidemiological or experimental data which link environmental hormone disruptors to reproductive cancers. Because, as has already been indicated, this information is inconclusive, deficiencies, inconsistencies, controversies and likely future developments will be discussed in the hope that they may stimulate and focus interest in this area and thus contribute in the longer term to answering the many important unanswered questions.

Hormones and reproductive cancers

Hormones have been linked with the risk, development and behaviour of a wide variety of human cancers, but the evidence is most convincing for malignancies of tissues of the reproductive systems of females and males which either produce, or are targets of, endocrine agents (Hawkins & Miller 1988). As is reviewed in more detail below, risk associations are usually drawn from epidemiological studies based on reproductive and menstrual factors, exposure to exogenous hormones and/or measures of hormone levels in women at high risk of cancer, including those who subsequently develop the disease. The corresponding evidence linking hormones with tumour behaviour is more compelling and founded on the presence of hormone receptors or end-point markers of hormone action within tumour specimens, the clinical observation of changes in tumour growth following endocrine treatment of patients with cancer, and the corresponding experimental findings in cell lines and xenograft models of human cancer. Some of these data will now be summarised in relation to breast, ovarian, endometrial, prostate and testicular cancers.

Breast cancer

The aetiology of breast cancer has a strong hormonal component. The disease occurs predominantly in females, in whom it is never seen before puberty (Boyle 1988). Risk is increased by a prolonged reproductive life (MacMahon *et al.* 1973), whereas a premature menopause is protective (Trichopoulos *et al.* 1972); e.g. castration at 35 years of age reduces subsequent breast cancer incidence to one-third of that in women undergoing a natural menopause. Whilst this illustrates the profound effect of ovarian hormones on the development of breast cancer, it also suggests that the disease occurs not simply as a result of exposure to steroid hormones - women at 35 will have already been exposed to over half the total amount of ovarian hormones produced during normal reproductive life. In this respect it is worth noting that pregnancy tends to protect against breast cancer (White 1987) despite being associated with high levels of circulating hormones. However, pregnancy does interrupt the cyclic pattern of trophic stimulation caused by regular menstrual cycles. Hence length and type of exposure may be equally important factors to consider when assessing the potential hazards of environmental oestrogens. Other risk factors for breast cancer appear to be associated with the postmenopausal period. For example, high bodyweight increases breast cancer risk in postmenopausal women (De Waard *et al.* 1977). This may be explained by the fact that, after the menopause, oestrogens are derived from extraglandular conversion of androgens (Vermeulen & Verdonck 1978), the rate and degree of which increases with bodyweight (James *et al.* 1981). Similarly, geographical differences in breast cancer, which show a marked increase in incidence in Western European countries and the USA compared with Asia (Fig. 1), are most pronounced in the postmenopausal period (Barber 1984); postmenopausal women in Western Europe and the USA tend to be more overweight than their Asian counterparts (De Waard *et al.* 1977) and this alone has been calculated to account for 80% of the disparity in breast cancer rates between Japanese and American women (Pike *et al.* 1993).

If exposure to hormones raises breast cancer risk, women given exogenous hormones should have an increased incidence of the disease. This has not been easy to prove. The most convincing evidence relates to the use of very high doses of diethylstilboestrol (DES) given to pregnant women. Subsequently, the daughters of these women had an increased incidence of clear-cell adenocarcinoma of the vagina and cervix (Greenberg *et al.* 1984) and there was a 35% increase in breast cancer in the mothers themselves (Colton *et al.* 1993). However, it took 20 years of follow-up before the hazards of breast cancer became apparent, emphasising the long latency period between exposure and appearance of disease symptoms. Potential dangers of less potent hormones have been even more difficult to identify. Thus the use of oral contraceptive pills comprising synthetic oestrogens and progestogens has been extensively studied over a protracted period of time yet most studies have failed to find significant increases in risk of breast cancer in women taking such preparations (La Vecchia 1992), although there are still concerns over long-term usage, especially when this occurs before first pregnancy or in young girls (Chilvers & Deacon 1994). Similarly, whilst the use of hormone replacement therapy (usually involving administration of conjugated oestrogens) at the menopause increases the incidence of endometrial cancer (Grady *et al.* 1992), effects on breast cancer risk are much less marked (Colditz *et al.* 1993, Beral *et al.* 1997). There are also several meta-analyses which have failed to

demonstrate a significantly increased risk of breast cancer in women on hormone replacement therapy, with the possible exception of those with a long duration of exposure (Steinberg *et al.* 1991).

Against this background it is perhaps not surprising that despite enormous research commitment it has proved difficult to demonstrate that circulating levels of hormones are related to the risk of breast cancer. Certainly the use of hormone levels to identify individuals or cohorts of women who have a substantial likelihood of subsequently developing the disease is presently impracticable (Miller 1996). A number of reasons have been used to explain these negative findings including (i) hormone abnormalities may be transient and only present at crucial inductive times, (ii) small differences in hormone levels which are difficult to detect become determinant if maintained over a long period of time, (iii) cyclicity and periodicity of hormone exposure is also influential, (iv) end-organ sensitivity to hormones is as important as hormone levels, and (v) cancer promotion is dependent upon particular fractions or combinations of hormones and tissue rather than circulating levels.

In contrast to the confusing situation with risk, the links between hormones and the progression of established cancers is much more compelling. Firstly, clinical experience shows that breast cancers may regress following endocrine deprivation therapy, most notably ovariectomy (Thomson 1902) and the administration of gonadotrophin-releasing hormone (GnRH) analogues (Blamey & Dixon 1991) in premenopausal women, and hypophysectomy (Luft *et al.* 1952), adrenalectomy (West *et al.* 1952), anti-oestrogens (Stewart 1989) and aromatase inhibitors (Miller 1997) in postmenopausal women. Conversely, there is evidence that administration of steroid hormones increases tumour growth (Dao *et al.* 1982, Conte *et al.* 1985). This may account for the observations from a prospective study of a large number of ostensibly normal women which showed that those who were diagnosed early in the follow-up period had higher levels of biologically available oestrogen than did controls (who did not develop cancer at the same time-period), whereas those who were diagnosed later did not (Bulbrook *et al.* 1986). This suggests that hormone levels relate to time of diagnosis rather than absolute risk and tumours appearing early may be stimulated to grow faster under the influence of raised hormone levels. Although several steroid classes have been shown to influence the growth of breast cancer cells under experimental conditions (Miller & Langdon 1997), the most influential seems to be oestrogen and this would reflect clinical experience. Thus all the endocrine treatment procedures have in common the ability to reduce tumour levels of oestrogen or antagonise its mechanism of action (Miller 1991), and major benefits of such therapy are associated with cancers which possess high affinity receptors for oestrogen (Hawkins *et al.* 1980). If, therefore, a search has to be made for environmental factors which might accelerate the growth of established breast cancers the logical place to start is amongst those with oestrogenic activity.

Ovarian cancer

Epidemiological studies support a role for hormones in the risk of ovarian cancer, as endocrine-related factors such as parity, length of reproductive life and oral contraceptive use all influence incidence of the disease (Beral 1987). The data associated with parity are striking - in comparison with nulliparous females, women with one or two children have half the risk, those with three or four children one-third the risk and women with five or more children are at a quarter the risk of developing ovarian cancer (Casagrande *et al.* 1979, Cramer *et al.* 1983). These observations, together with the finding that oral contraceptive use also decreases risk (Rosenblatt *et al.* 1992) have led to the proposal that ovulation promotes ovarian cancer by causing trauma to the surface epithelium of the ovary, repair of which induces rapid cellular proliferation, enhancing the likelihood of cancer (Fathalla 1971, Godwin *et al.* 1992). Indirect support for this suggestion has come from the report of increased incidence of ovarian cancer in infertile women whose ovaries have been hyperstimulated to produce multiple ovulations (Whittemore 1993). The implication of such epidemiological studies is that a hormone environment which encourages ovulation or ovarian proliferation will increase risk of subsequent ovarian cancer.

The involvement of hormones in the behaviour of established ovarian cancers is underpinned by the finding of hormone receptors in such tumours. Thus oestrogen receptors (ER) have been found in 60% of ovarian cancers (Slotman & Rao 1988). The presence of steroid receptors may also be associated with a more favourable prognosis (Kieback *et al.* 1993); that this is associated with a direct hormone influence is supported by studies in cell lines of human ovarian cancers which show that the growth of cells with steroid receptors is likely to be influenced by steroid hormones (Langdon *et al.* 1997). In this respect it is relevant that clinical studies have shown that endocrine manipulation, for example treatment with anti-oestrogen (Myers *et al.* 1981, Ahlgren *et al.* 1993) or progestins (Timothy 1982, Fromm *et al.* 1991), may produce beneficial effects in patients with ovarian cancer, although response rates are usually low (approximately 10%).

Endometrial cancer

Epidemiological studies suggest that hormones, in particular oestrogens, are involved in the development of endometrial cancer; risk factors include oestrogen use,

oestrogen-containing oral contraceptives, anovulation, nulliparity and obesity (King 1997). Prolonged use of tamoxifen, which although marketed as an anti-oestrogen has partial oestrogen agonist activity on normal endometrium, is also associated with increased incidence of endometrial cancer (Satyaswaroop & Tabibzadeh 1997). Interestingly, progestin supplementation seems to protect against the promoting effects of oestrogen in both contraceptive (Schlesselman 1991) and hormone replacement preparations (Richardson & MacLaughlin 1978). These observations have led to a hypothesis of 'unopposed oestrogen' in which endometrial cancer results from increased exposure to either endogenous or exogenous oestrogen without a parallel excess of progestin (Key & Pike 1988).

Both oestrogens and progestin appear to be involved in the growth and differentiation of established endometrial cancers (King 1997). Positive correlations have been found between the presence of ER and progesterone receptors and degree of tumour differentiation (McCarty *et al.* 1979); most well-differentiated tumours possess both steroid receptors whereas only a small proportion of undifferentiated tumours do. The presence of steroid receptors is also associated with a good prognosis (Creasman 1993). In this respect it is relevant that treatment with either tamoxifen or progestins may produce clinical benefits in patients with endometrial cancer (Rendina *et al.* 1984, Lentz 1994).

Prostate cancer

Steroid hormones are considered crucial for the development of prostate disease including cancer. However, most evidence is circumstantial and again largely based on aetiology. Tumours of the prostate have not been recorded in men castrated before puberty (Lipsett 1979) and a reduction in risk is seen in men with hepatic cirrhosis (Robson 1966), presumably on account of reduced circulating levels of androgens. The two-fold higher incidence of prostate cancer in black, compared with white, men in the USA (Fig. 1) is also associated with higher total and free testosterone levels in young black, compared with white, men (Ross *et al.* 1986). However, it has proved difficult to obtain definitive evidence that elevated circulating levels of hormones are associated with increased risk, although a recent case-control study in men prior to cancer diagnosis suggested higher levels of circulating testosterone and lower levels of oestradiol and sex hormone-binding globulin (SHBG) exist in those men subsequently developing the disease (Gann 1996). The elevation in testosterone was modest and values were well within the normal range, and it may be again that the prolonged presence of androgens is more important than high levels or that exposure at critical times, such as *in utero*, adolescence or old age is crucial.

The suggestion that *in utero* or perinatal exposure of the developing male to hormones may be influential in subsequent development of prostate cancer comes from a number of observations. These include relationships to factors such as birthweight, prematurity and pre-eclampsia, which in turn can be interpreted as reflecting different hormone levels and exposures prenatally (Tibblin *et al.* 1995, Ekbom *et al.* 1996). More direct support for this line of thinking comes from the observation that the blood levels of testosterone are higher among black than white pregnant women in the USA and the male offspring of the former are at twice the risk of developing prostate cancer compared with white males (Henderson *et al.* 1988, Ross *et al.* 1992). It has been argued that perinatal oestrogen exposure of the human and animal prostate may be important in predisposing the prostate to cancerous changes in later life (see Santti *et al.* 1994, Salo *et al.* 1997), though conclusive evidence to support this possibility is currently lacking. A recent study has shown that modest elevation of maternal oestrogen levels in mice can result in hyperplasia of the prostate in the male offspring in adulthood, although oddly, more extreme elevation of oestrogen levels had the opposite effect (vom Saal *et al.* 1997). As the many males whose mothers were exposed to DES *in utero* reach the age range when prostate cancer emerges it will prove of particular interest to see whether or not they exhibit an altered incidence of this disease. It is clear that the prostate is an important site of action of both androgens and oestrogens (Thomas & Keenan 1994) in animals and man throughout life (Cunha *et al.* 1987) and, as has been argued by others (Walsh 1988, Santti *et al.* 1990, 1994), it may be that the balance between androgens and oestrogens at critical times determines subsequent risk of prostate cancer.

In terms of behaviour of established cancers, reduction of testosterone to castrate levels may result in tumour regression. This may be achieved by surgical castration (Huggins & Hodges 1941), GnRH agonists (Debruyne 1989) or pharmacological doses of oestrogen (The Veterans Administration Cooperative Urological Research Group 1967).

Testicular cancer

Testicular cancer is primarily a disease of young men (15-45 years of age) and it is now the commonest cancer of young men in most developed countries. In virtually all countries in which there is a good cancer registry, the incidence of testicular cancer is increasing progressively (Fig. 1) and may be doubling in incidence as rapidly as every decade (Toppari *et al.* 1996). Greater than 95% of testicular tumours are germ cell derived, and though their

aetiology is still poorly defined, it is clear that many, if not all, of these could have their origins in early (probably intrauterine) development (Toppari *et al.* 1996). This conclusion has been reinforced by recent studies of the differences in incidence of testicular germ cell cancer between north European countries, which highlighted the difference in risk of birth cohorts; later year of birth was associated with a progressively increasing risk irrespective of whether the country had a high or a low incidence (Adami *et al.* 1994).

Prominent among established risk factors for developing testicular germ cell cancer are conditions known to be associated with abnormalities in hormone action in the foetus, such as cryptorchidism, hypospadias, inguinal hernia and intersex/androgen-resistant syndromes (Savage & Lowe 1990); the last are at particularly high risk of developing testicular cancer. Conversely, in the USA black men are at considerably lower risk of developing testicular cancer than are the corresponding white men (Spitz *et al.* 1986) (Fig. 1), and it is established that black women have higher circulating levels of androgens during pregnancy than do white women (Henderson *et al.* 1988, Ross *et al.* 1992). There is suggestive but inconclusive evidence from case-control studies that exogenous hormone exposure (including oestrogens) during pregnancy is associated with increased risk of male offspring developing testicular cancer in adulthood (see references and analysis in Toppari *et al.* 1996). It is well established that oestrogen (particularly DES) administration to women or animals during early pregnancy results in an increased incidence of cryptorchidism and/or inguinal hernias (Morrison 1976, United Kingdom Testicular Cancer Study Group 1994, Toppari *et al.* 1996), and the latter can also be induced in transgenic mice which overexpress ERα (Davis *et al.* 1994). As cryptorchidism and inguinal hernia are themselves both risk factors for testicular cancer, any effect of intrauterine exposure of the male to oestrogens on testicular cancer risk may be indirect, resulting for example from suppression of androgen production and/or action (see Majdic *et al.* 1996).

Summary: hormones and human reproductive cancers

Lessons can be learned from links between hormones and the natural history of cancer. Whilst the aetiology of certain cancers may have a strong endocrine component, it has not been easy to demonstrate unequivocal associations between either endogenous or exogenous hormones and the development of cancer. Amongst the explanations for this are (i) small differences in hormone levels, which may be within normal ranges and difficult to detect, may be influential if maintained over a sustained period, (ii) transient critical inductive periods of susceptibility, (iii) patterns of exposure and combinations of hormones are more important than individual levels, (iv) differences in tissue sensitivity (including factors which determine hormone levels within tissues), and (v) the time between exposure and appearance of cancers may be long, demanding an extended follow-up. Many of these same considerations will have to be taken into account when assessing the potential impact of environmental chemicals on the natural history of cancer.

Hormonally active environmental chemicals

In the context of this review, the term ‘environmental hormone’ has been used to describe any chemical to which humans are exposed which originates from outside the body and which either has intrinsic steroid hormonal activity and/or when ingested or absorbed into the body has the potential to alter endogenous steroid hormone levels. This definition thus includes ‘recycled’ oestrogens which may have been produced initially within the human body but which eventually find their way back to man via the environment. There are three distinct types of environmental hormones to which humans are exposed (Fig. 2): (i) non-steroidal, industrial or man-made chemicals, (ii) naturally occurring plant and fungi-derived oestrogens, and (iii) steroidal/synthetic oestrogens. Each of these is considered separately below with an outline of what is currently known about the potential routes and level of human exposure.

Industrial chemicals

Concerns about potential adverse effects of environmental hormones on human health in recent years have originated largely from publications demonstrating that several widely used environmental chemicals are weakly oestrogenic. What may surprise many readers is that this is not new information but is essentially the ‘rediscovery’ of what had already been observed around 60 years ago when Dodds and Lawson (1936) reported that a number of man-made chemicals were oestrogenic *in vivo* in the rat uterine weight bioassay. Indeed it was such findings which led Dodds to the discovery of DES, which he selflessly refused to patent in order that mankind could derive widespread and non-costly benefits from its usage. As it turned out, therapeutic use of DES was anything but beneficial, as is reported elsewhere in this review. Unfortunate though this may be, the administration of very high doses of DES to some 7 million pregnant women worldwide (Palmlund 1996) at least offers us a ‘worse case scenario’ of the potential adverse effects of exposure of the developing foetus to abnormally high oestrogen levels and provides a yardstick against which to estimate the

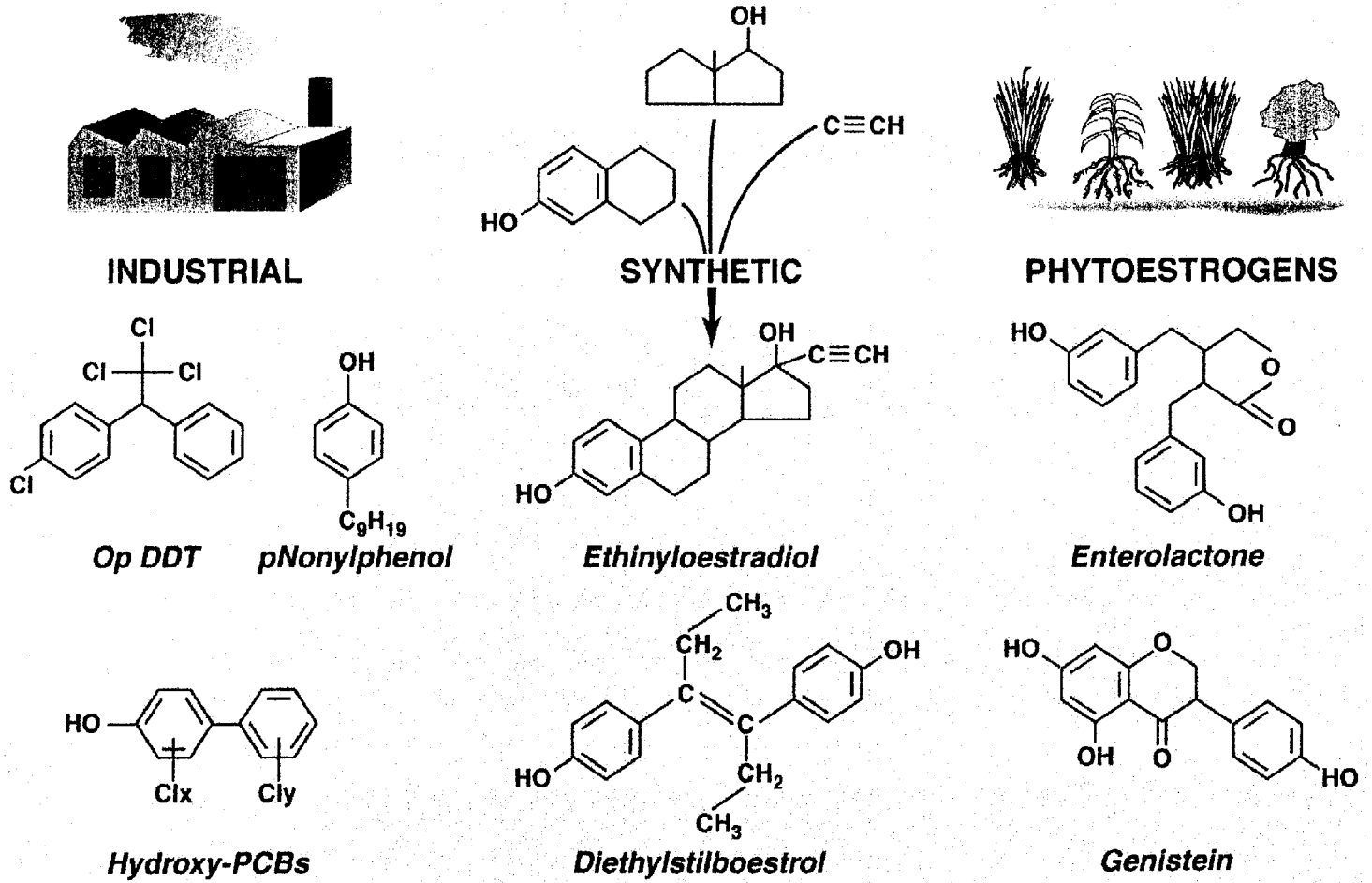

Figure 2 Examples of the three main types of oestrogenic compounds to which humans are exposed via their environment, namely industrial chemicals/pesticides (left), synthetic steroidal or non-steroidal oestrogens (centre) and naturally occurring plant oestrogens (phytoestrogens; right). Note that (i) other than the presence of one or more phenolic rings, there is no particular consistency in chemical structure between the different compounds, and (ii) the biosynthetic route is a diagrammatic representation rather than an actual transformation.

potential adverse effects of the many-fold weaker 'environmental oestrogens'.

Chlorinated chemicals (organochlorine pesticides/ polychlorinated biphenyls/dioxins)

Various chlorinated pesticides, including the *o′,p′*-isomer of DDT, were shown many years ago to be weakly oestrogenic *in vivo* (Tullner 1961, Bitman *et al.* 1968, Bitman & Cecil 1970, reviewed in Nelson *et al.* 1978, Toppari *et al.* 1996) and this has been confirmed and extended by *in vitro* assays more recently (e.g. Korach *et al.* 1988). These chemicals have the general property of being lipophilic and persistent in the environment, which is one reason why they are such effective pesticides. As several of the organochlorine pesticides, or their metabolites, tend to accumulate in body fat, and human exposure may be substantial in some cases (see Kelce & Wilson 1997 and below), chemicals of this type have to be given serious consideration in the context of human cancer induction. The lipophilic nature of organochlorine pesticides means that they tend to concentrate in the food chain and therefore animals at the top of the food chain, such as man (Fig. 3), are likely to be more highly exposed (Toppari *et al.* 1996). DDT is the most notorious example, and consideration of how humans were exposed during its first usage back in the 1940s and 1950s is today quite astonishing.

When first discovered, DDT was hailed as little short of miraculous and was classed as one of the Allies' war weapons during the Second World War. This was because of its ability to eradicate all manner of insect pests which transmitted diseases such as typhoid, malaria etc. It was used in ways which even for today's more user-friendly pesticides would not be contemplated (Sharpe 1995). Nowadays, human exposure to pesticides is minimised and regulated whereas DDT was actually applied to people! In the war, whole populations of cities were dusted with DDT powder or sprayed from aeroplanes as we now spray crops, whilst soldiers were issued with DDT-impregnated shirts. After the war, it was sprayed on beaches and in housing suburbs, with children running in and out of the spray for fun. It was even used in household fly-sprays, including in the UK. In view of its widespread usage, its lipophilic nature and its persistence (DDT has a half-life in the body and the environment of 60-100 years), it is realistic to conclude that human exposure was substantial and widespread during the period 1940-1960 (Kelce & Wilson 1997). This is echoed by the fact that virtually everybody in the Western world, irrespective of their age, has detectable DDT and/or its metabolites in their body fat despite the fact that the use of DDT has been banned in Western countries for over 20 years. Usage of DDT in developing countries is still on the increase and the worldwide production of DDT is higher today than at any time previously (Sharpe 1995). As DDT is easily transported via air and produce around the world, it still poses a potential risk to Western countries today.

As there is growing evidence that predisposition to develop a hormone-dependent cancer of the reproductive system in adulthood may be induced during 'critical periods' in foetal or infant life (see above), of particular concern is the mobilisation of DDT stored in fat by breastfeeding mothers and its transfer via milk to their babies (Rogan *et al.* 1986, Somogyi & Beck 1993) (Fig. 4). Levels of DDT and its isomers in breastmilk have therefore been carefully monitored over the past two to three decades and although it is reassuring that levels are continuing to decline in Western countries (Somogyi & Beck 1993), there can be little doubt that infants in the first year of life were probably exposed to particularly high levels of DDT during the period 1945-1965 (Lang *et al.* 1951, Rogan *et al.* 1986). These concerns are reinforced by recent data showing that women born in countries in which DDT is still used (e.g. Mexico and countries in the developing world), and who subsequently become resident in the USA, have substantially higher breast milk levels of DDT/DDE than USA-born women (Kelce & Wilson 1997, Marien 1997). Even though the past 30 years or so have witnessed a major decline in breastfeeding in Western countries, cow's milk and cow's milk formula will also contain reasonably high levels of DDT/DDE. The fact that breastfeeding leads to mobilisation of fat stores, and thus of any lipophilic chemicals stored in it, may also be important in terms of the risk that these chemicals pose to the breast and the development of breast cancer. Breastfeeding will lower exposure of the breast to such chemicals whilst women who bottlefeed will presumably retain all of these chemicals within their breast tissue (Fig. 4).

Several organochlorine pesticides such as kepone and dieldrin, which are essentially derivatives of DDT, share its properties of persistence and lipophilicity to a variable extent. Several of these pesticides have been shown to be oestrogenic *in vivo* and/or *in vitro*, although they have not been used in as widespread a manner as DDT (Gellert *et al.* 1972, Kupfer 1975, Toppari *et al.* 1996).

Other man-made chlorinated chemicals which are highly persistent and to which there has been substantial human exposure are the polychlorinated biphenyls (PCBs). These comprise a complex mixture of congeners, some of which have been shown to possess either oestrogenic or anti-oestrogenic properties (Bitman & Cecil 1970, Jansen *et al.* 1993, Netsaretnam *et al.* 1996, Toppari *et al.* 1996). PCBs are hydroxylated in animals and the resultant hydroxybiphenyls are more potent oestrogens *in vitro* than the native PCBs (Korach *et al.* 1988). PCBs were used extensively in the electrical industry as dielectric fluids in transformers and capacitors, as heat

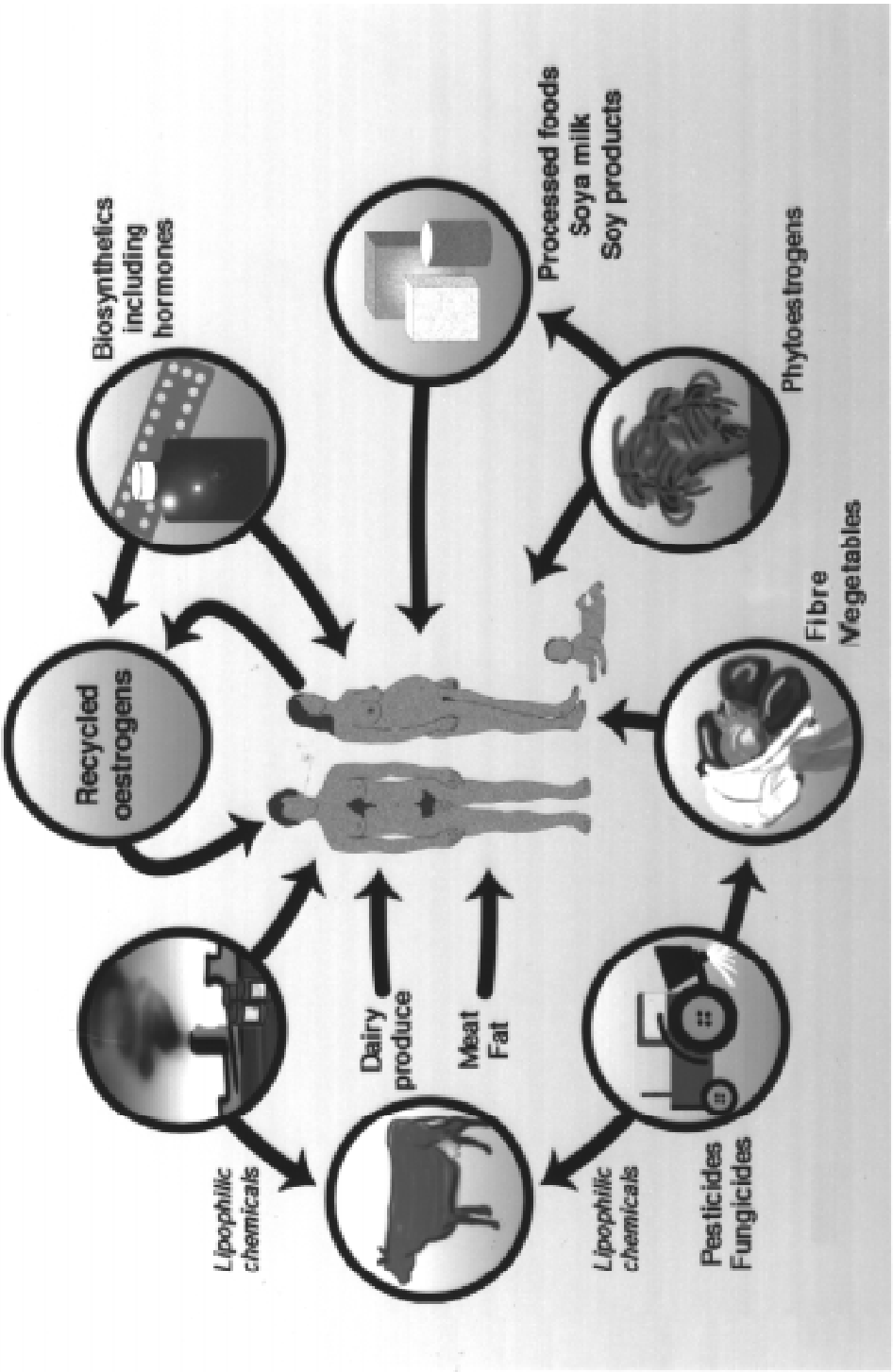

Figure 3 Diagrammatic representation of the many different routes via which humans are exposed to hormonally active chemicals via their environment. As man is at the pinnacle of the food chain, we are particularly at risk from lipophilic chemicals which bioaccumulate in fat, and there are numerous examples of hormonally active lipophilic chemicals to which we have been exposed over the past 50 years (e.g. DDT). Differences in dietary fat content and in body fat burdens between Western and Oriental countries are known to be important in the aetiology of reproductive cancers (see text).

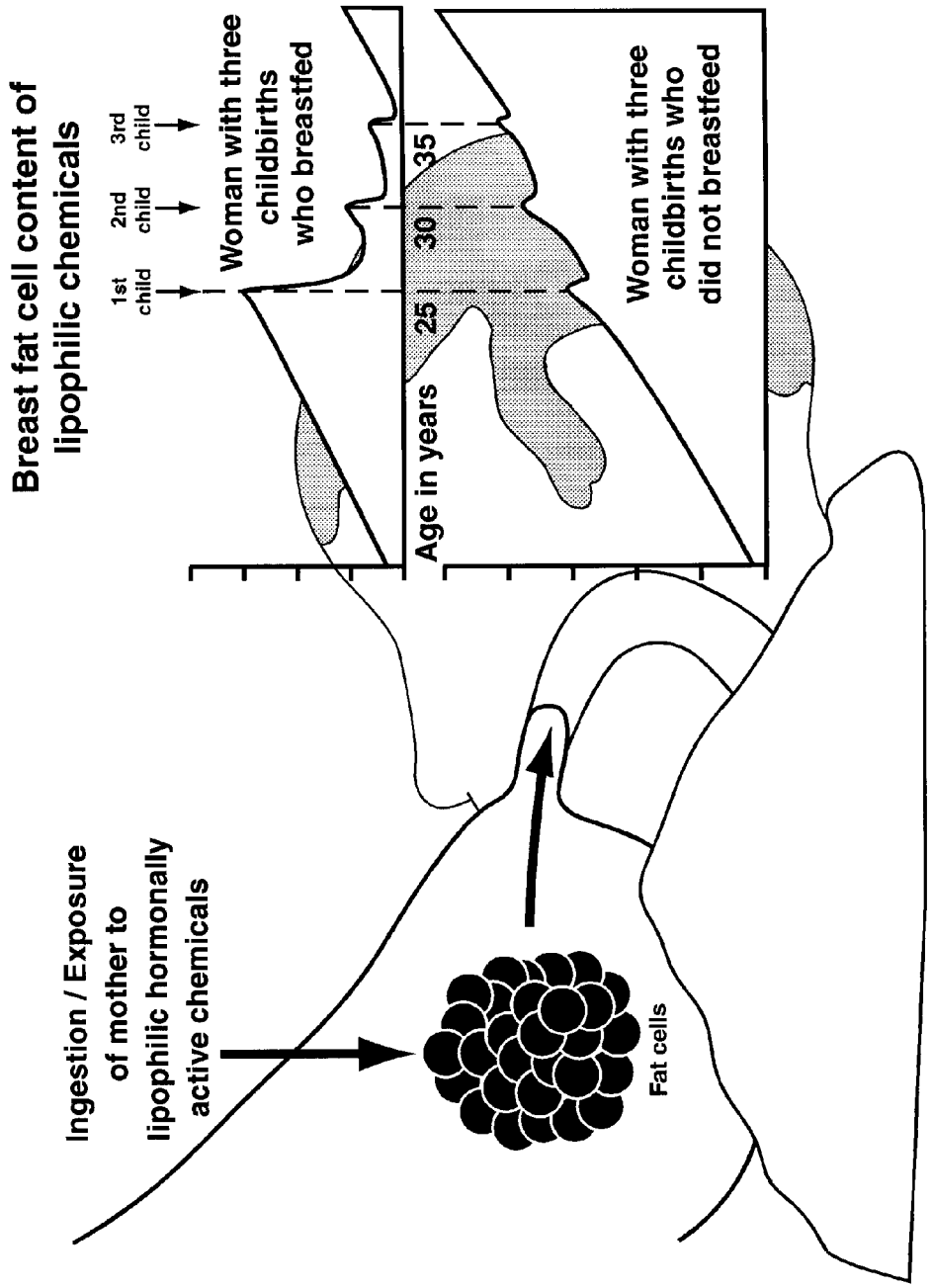

Figure 4 Diagrammatic representation of the likely impact of breastfeeding on the levels in breast fat of lipophilic chemicals which have accumulated there over the years prior to first pregnancy. In a woman who breastfeeds her babies, mobilisation of breast fat stores is likely to reduce the concentration of lipophilic chemicals (and deliver them to the infant) whereas this will not occur in a woman who does not breastfeed her baby. In theory, the breasts of a woman who has not breastfed will therefore be exposed to much higher endogenous levels of lipophilic chemicals for a much longer period than will the breasts of a woman who has had several children and has breastfed them all. Changing patterns of breastfeeding over the past half century in Western societies coupled with the widespread usage of lipophilic industrial/agricultural chemicals could therefore have resulted in increased lifetime exposure of the breast to lipophilic hormonally active chemicals.

exchange and hydraulic fluids, as flame retardants and in some adhesives and waxes. As with DDT, one of the primary problems with PCBs is their persistence in the environment. Human exposure to PCBs has been reasonably substantial in the recent past, though the complexity of PCB structure and the resultant differences in oestrogenicity/anti-oestrogenicity make it extremely difficult to predict what, if any, biological effects might be caused (Safe 1995, Toppari *et al.* 1996).

Other chlorinated chemicals which are recognised as being extremely toxic are the dioxins/furans, the best known of which is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin). These are not made intentionally but are by-products of the manufacture of organochlorine compounds and their incineration, including by the petrol engine. Dioxins have been shown to exert a range of effects on both the development and function of the reproductive system (Petersen *et al.* 1993), effects which are thought to occur primarily via interaction with the aryl hydrocarbon receptor, the physiological functions and ligands for which are unclear (Whitlock 1994). The resulting effects of dioxins in a wide range of animal models and isolated cell systems can be classified as being anti-oestrogenic and/or anti-androgenic (Petersen *et al.* 1993, Safe 1995, Toppari *et al.* 1996). The furans/dioxins are lipophilic and tend to concentrate in body fat. The increase in consumption of animal fats in the Western world over the past half-century, coupled with the widespread use of organochlorine chemicals, therefore means that human body burdens of furans/dioxins may have increased in this time-frame (Fig. 3), though there is no clear evidence that biologically significant levels have been achieved. There have been industrial accidents in which high-level human exposure to dioxins has occurred (e.g. Seveso) and the possible effect of such exposure on cancer risk is discussed below.

The oestrogenicity of organochlorine pesticides and PCBs has been recognised for at least one to two decades, but there have been some more recent developments. Perhaps most important has been the discovery that the principal and persistent metabolite of DDT in the body, DDE, has potent anti-androgenic activity (Kelce *et al.* 1995, Kelce & Wilson 1997). Moreover, some PCB congeners have been shown to possess anti-thyroidal activity (Gray *et al.* 1993, Ness *et al.* 1993).

Alkylphenolic compounds

The original description of the oestrogenicity *in vivo* of certain alkylphenolic compounds was as long ago as 1938 (Dodds & Lawson 1938), but they were subsequently 'rediscovered' some 40 years later based on their competition for binding to the ER *in vitro* (Mueller & Kim 1978). Subsequently, it was demonstrated that nonylphenol leaching out of plasticware (Soto *et al.* 1991) had oestrogenic effects on cells and *in vivo*. Since then several alkyphenolic compounds have been shown to be oestrogenic *in vitro* and *in vivo* in a number of mammalian and non-mammalian test systems for oestrogenicity (Soto *et al.* 1991, White *et al.* 1994, Bicknell *et al.* 1995, Jobling *et al.* 1996, Lee & Lee 1996) and the relationship between oestrogenicity and the position and branching of the alkyl group has been established using an *in vitro* oestrogen screening system (Routledge & Sumpter 1997). Alkylphenols comprise a large group of chemicals which has been in use for over 40 years. They are classed as being non-ionic surfactants (i.e. surface-active or wetting agents, emulsifiers or detergents), which have many and varied industrial uses, most notably as industrial detergents, for example in the wool industry. They are also used in some household detergents, though this is much more prevalent in countries outside the UK and Europe such as the USA. Another less well known but major use of alkylphenols is in plastics as an anti-oxidant to prevent discolouration of the plastic by sunlight, a process which involves gradual release of free alkyphenolic compound, such as nonylphenol, from the plastic. Other uses of alkylphenols are as petrol additives ('the petrol that cleans your engine'), as spermicides in condoms, in sprays for delivering pesticides and uses in some shampoos/cosmetics.

Production of alkyphenolic compounds is measured in millions of kilograms annually, of which some 60% find their way into the aquatic environment, where they are recognised as being harmful to life, though probably more because of their detergent properties rather than because of their oestrogenicity. Alkylphenols are present in river water in concentrations varying from <0.6 to 45 µg/l (Nimrod & Benson 1996) and have also been detected at lower concentrations (~20 ng/l) in drinking water (Clark *et al.* 1992). At these levels, it is probably unlikely that any significant effect on man would occur. Our level of exposure to alkylphenolic compounds via other routes (i.e. leaching from plastics, absorption from contact with detergents, shampoos, cosmetics) is unknown, but is likely to be more substantial than our exposure via tapwater.

Phthalate esters

Two of the many phthalate esters (butyl benzyl phthalate (BBP) and di-*n*-butyl phthalate (DBP)) were shown initially to be weakly oestrogenic in *in vitro* tests (Jobling *et al.* 1995), but subsequent screening of a larger range of phthalates, including those which are most widely used, has revealed that most are non-oestrogenic *in vitro* screens (Harris *et al.* 1997). This class of compound is amongst the most ubiquitous of all man-made chemicals in the environment. Phthalates are literally everywhere and ingestion by humans is both unavoidable and substantial, being in the range of 2-6 mg per person per day. Phthalates

are plasticisers, i.e. they lend durability and flexibility to plastics such as PVC though they also have many other uses (e.g. lubricating oils, some adhesives, printing inks, perfumes). Like many chemicals added to plastics (e.g. alkylphenols described above), phthalates are not an integral part of the finished material and can therefore leach out over time. This explains, firstly, why plastic tends to become brittle with time and, secondly, why phthalates are so widespread (MAFF 1987). Because plastics in one form or another are now an integral part of our everyday lives and have been used extensively in wraps or containers for much of our foodstuffs and drink (though this has changed appreciably over the past decade or so), it is not surprising that phthalates are easily detectable in most foodstuffs (MAFF 1987), in particular dairy products, in which levels of phthalates tend to parallel fat content (Page & Lacroix 1992, Sharman *et al.* 1994). Lower levels of phthalates have been reported in formula baby milk powders (MAFF 1996).

Based on human intake of the two identified oestrogenic phthalates (BBP, DBP), it seems unlikely that the levels ingested would be sufficient to exert oestrogenic effects on their own. However, intriguing recent data show that DBP can exert pronounced anti-androgenic effects on male rat pups following exposure of the mother during gestation and lactation (Wine *et al.* 1997), although these effects only occurred with high frequency after high levels of exposure (794 mg/kg per day). Unpublished data from *in vitro* tests for anti-androgenicity appear to confirm these findings (J P Sumpter, personal communication; P M D Foster, personal communication). This development is of significance in view of the evidence presented above implicating disorders of androgen production or action in the aetiology of testicular cancer.

Bisphenol-A and derived compounds

Bisphenol-A is weakly oestrogenic *in vitro* and *in vivo* in a number of test systems (Bitman & Cecil 1970, Krishnan *et al.* 1993) and was one of the compounds identified as being oestrogenic *in vivo* nearly 60 years ago by Dodds & Lawson (1936). This class of compound has many and varied uses. Like several of the other chemicals described above, it is an ingredient of certain types of plastic (polycarbonates) and is also used in false teeth and teeth sealants, in acrylic resins, in photocopying, certain fungicides and in the lacquer coat lining of tinned food cans. Leaching of bisphenol-A from all of these products is likely and has been documented for polycarbonate plastic (Krishnan *et al.* 1993), tin cans (Brotons *et al.* 1995) and teeth sealants (Olea *et al.* 1996). The level of human exposure to bisphenol-A from these various sources remains unknown and it can only be speculated whether or not it is sufficient to cause any biological effects.

Tributyl tin (TBT)

TBT has been used widely since the early 1950s as an anti-fouling agent on marine vessels, i.e. it is painted on hulls and prevents the attachment of barnacles etc. The leaching of TBT from the hulls of boats is now established as being the cause of a condition termed 'imposex' in certain marine gastropods (whelks etc.), especially those in estuaries (Bryan *et al.* 1986, Oehlmann *et al.* 1996), although it has also been reported recently in the middle of the North Sea (Ten Hallers-Tjabbes *et al.* 1994) and is clearly a worldwide phenomenon (Ellis & Pattisina 1990). In this condition, the females grow a penis, which interferes with normal mating and hence reproduction. It appears that the primary mechanism by which TBT induces this effect is via the inhibition of aromatase, which elevates androgen levels thus causing permanent overgrowth of the normally vestigial penis in the female (Spooner *et al.* 1991, Oehlmann *et al.* 1996). As a result of these effects, which have exerted enormous impact on gastropod populations in many sites in the world, most countries have banned or restricted the use of TBT as an anti-fouling agent. However, TBT is a highly effective biocide and has found increasing use on land as a preservative for wood and other materials, in textiles, polyurethane rigid foams, paints and adhesives and as a plastic stabiliser.

The levels of exposure of humans to TBT, and whether or not this could induce biological effects, are unclear. However, there are three reasons for concern. First, TBT is extremely toxic (=effective) and exerts its effects on gastropods at concentrations in sea-water of ~10 pg/ml (Oehlmann *et al.* 1996). Secondly, like many of the other chemicals referred to above, it has many and varied uses, some of which could theoretically lead to significant human exposure. Thirdly, it is a man-made chemical which was only discovered to have the ability to alter the activity of aromatase in gastropods after a 'catastrophic environmental event'. We should therefore not forget that other chemicals to which we are exposed might have similar properties of which we are currently unaware.

Other chemicals

Although it is generally accepted that oestrogenicity of a chemical cannot be predicted from its chemical structure, chemicals which contain one or more phenolic rings are potentially likely to be oestrogenic, either in their native form or after metabolism. This is important because in excess of half of all industrial chemicals (which are numbered in tens of thousands) would fall into this category and measurement of 'environmental pollution', for example in waterways, often relies simply on the measurement of phenolic compounds. Thus, it is not unreasonable to predict that further oestrogenic man-made chemicals will be identified and will contribute to the

overall burden of human exposure to environmental oestrogens (Fig. 3). In this context it is possible that (i) the chemicals identified so far as being oestrogenic represent the tip of the iceberg, and (ii) chemicals which are oestrogenically more active than those listed above, or to which human exposure is more extensive, will subsequently be discovered.

A further development which needs to be kept in mind is the surprising discovery that a number of the recently identified oestrogenic chemicals (some of the phthalates and alkylphenols) may also be anti-androgenic *in vitro* (W R Kelce, personal communication; J B Sumpter, personal communication). It remains to establish how widespread this 'duality' of action is amongst the oestrogenic chemicals, but the fact that oestrogenic and anti-androgenic isomers of DDT also exist is perhaps significant. In biological terms the actions of androgens and oestrogens are often opposite to each other, which means theoretically that oestrogenic and anti-androgenic activity deriving from the same molecule might increase the chances of such compounds interfering with development or function of reproductive tissues. In this regard, impairment of androgen action *in utero* is clearly a serious risk factor for the development of testicular cancer (see above) and there is evidence to suggest that androgens antagonise the proliferative actions of oestrogens on the human breast (see Kelce & Wilson 1997).

Phytoestrogens and mycoestrogens

Over 300 plants and fungi naturally produce compounds which have oestrogenic activity (Verdeal & Ryan 1979) and this has been known for decades (Bradbury & White 1954). Indeed, in terms of *in vitro* oestrogenic potency these compounds are considerably more active than most of the oestrogenic industrial chemicals. Human exposure to phytoestrogens, and to a lesser extent to mycoestrogens, can also be substantial (Setchell 1985, Adlercreutz 1995). The richest dietary source of phytoestrogens for humans is soya and soya-derived products (Fig. 3), although legumes, whole grains and flax-derived products are also potentially important, especially in vegetarians (Knight & Eden 1996). The oestrogenic potency of these natural compounds is illustrated by the numerous instances in which ingestion severely disrupts normal reproduction in animals such as sheep ('clover disease'), pigs ('mouldy corn syndrome'), captive cheetahs and quail (see Kaldas & Hughes 1989, Setchell 1995), effects which can sometimes be irreversible. Based on such findings, it has been suggested that phytoestrogens have been selected by plant species during evolution since they may offer long-term protection by reducing the reproductive efficiency of their predators.

Phytoestrogen exposure may also have effects in humans. This evidence ranges from the largely anecdotal stories of disrupted menstrual cycles in women when picking hops (which contain phytoestrogens) to the well-controlled study of menstrual cycle length and reproductive hormone levels in women when placed on a high (soya) isoflavone-containing diet (Cassidy *et al.* 1994). The latter study showed that the isoflavonoid phytoestrogens prolonged the menstrual cycle in normally cycling women by suppressing follicle-stimulating hormone levels and thus prolonging the follicular phase of the cycle.

There are also a number of observations which relate dietary exposure of humans to phytoestrogens and to hormone-related cancers. In Western societies, the decline in consumption of vegetables and fruit (and thus of fibre) over the past 30-40 years would have reduced exposure to phytoestrogens. This change coincides with increased prevalence of breast cancer (Adlercreutz 1995). More recently, the cheapness of soy beans as a source of protein has led to the inclusion of soy-derived protein/flour in many processed foods - latest estimates suggest that ~60% of all processed foods now contain soy flour/protein (Fig. 3). As the isoflavonoid phytoestrogens are associated with protein/flour, and not with the fat/oil, derived from soy beans, exposure of Western societies to phytoestrogens is likely to be on the increase. Whilst dietary change may be argued as being beneficial, e.g. by lengthening the menstrual cycle (see below), it is somewhat disturbing that compounds with hormonal activity can be added (inadvertently) to foods in a wholesale manner without the requirement for proof of safety (or benefit), especially in view of the known involvement of oestrogens in chronic disease/cancer. There is one particularly extreme example of this which has recently come to light - soy formula milk for babies.

In the last two to three decades, the feeding of soy formula milk to infants as a substitute for breastfeeding/cow formula milk has increased progressively and, in many Western countries, 10-20% of infants are now reared this way. This has been presumed safe in view of the evidence for health-beneficial effects of soy in Asian diets, but a recent study (Setchell *et al.* 1997) has shown that infants fed on a 100% soy formula milk diet have blood levels of isoflavonoid phytoestrogens which are nearly 1000-fold higher than those in Asian infants breastfed by a mother who is consuming a soy-rich diet. The effects of such high exposure to phytoestrogens during infancy are essentially unknown but cases could be made for this having both beneficial effects, in terms of future cancer risk (prostate, breast), or detrimental effects in terms of reproductive development (see Clarkson *et al.* 1995, Setchell *et al.* 1997); however, these possibilities are based on animal studies or epidemiological data for

adults (see below) and should therefore be viewed with circumspection.

Steroidal/synthetic oestrogens

Human exposure to steroidal/synthetic oestrogens can be divided into two categories: (i) exposures from intentional ingestion, either premenopausally from taking an oestrogen-containing oral contraceptive or postmenopausally from hormone-replacement therapy, and (ii) unintentional ingestion/absorption from foodstuffs, drinking water or cosmetic products. In (i), perhaps the most important consideration is the dramatic reduction in oestrogen content of oral contraceptives since their inception back in the 1960s with the result that women in different age cohorts have widely differing exposures, a factor which has been addressed in numerous epidemiological studies relating to risk of breast and other cancers of reproductive tissues in women (see above).

The level of exposure of man to steroidal/synthetic oestrogens via unintentional ingestion is poorly understood, but again there are likely to be large inter-individual differences as well as differently exposed age cohorts. The principal potential routes of such exposure (Fig. 3) are (i) ingestion of growth-promoting oestrogens in meat/poultry, (ii) ingestion of oestrogens in cow's milk and milk-derived products, (iii) ingestion of 'recycled' oestrogens in drinking water, and (iv) absorption via the skin of oestrogens present in cosmetics/shampoos.

(i) Because relatively low doses of oestrogens have growth-promoting properties and preferentially increase lean tissue, oestrogens have been used extensively in the rearing of livestock for meat, including poultry, beef cows and pigs (Lamming 1957, 1984). For around 20-30 years in Europe, orally active oestrogens such as DES were used widely for this purpose until their use was banned in 1981 because of concerns about the risk to man from residues in meat (Anon 1982, Lamming 1984, Hoffman & Evers 1986). Since this time, only orally inactive steroidal oestrogens or naturally derived products such as mycoestrogens (e.g. zearalone) are permitted and these are used almost universally. Although codes of practice for the use of growth promoters such as DES were available, it is unclear how strictly these were enforced, which means that the level of human exposure in the 1950s-1970s is difficult to estimate (Anon 1982). Based upon various reports in the medical literature in which overt symptoms of oestrogen exposure in prepubertal children are described, and attributed (though not proven) to use of DES in livestock (Fara *et al.* 1979, Anon 1982, Comas 1982, Rodriguez & Toro-Sola 1982), it is most likely that exposure to DES via meat at levels sufficient to cause clinically obvious symptoms occurred only in isolated instances involving relatively small numbers of individuals. However, as DES is extremely potent and does not bind to SHBG (Sheehan & Young 1979), the possibility that lower level chronic exposure to DES occurred in the population at large, as the result of residues in meat/poultry, cannot be dismissed with confidence.

(ii) Changes in dairy farming practices since the Second World War have meant that when dairy cattle get pregnant they continue to produce and give milk. Because oestrogen levels in cows increase during pregnancy, as in man, and transfer of some of this oestrogen to milk occurs (Holdsworth *et al.* 1982, Hamon *et al.* 1990), this means that cow's milk now contains more oestrogen (mainly in the form of oestrone sulphate) than was the case 50 years ago. As consumption of dairy products has increased over this time period in Western societies, this may have contributed to the overall oestrogen burden of individuals. Transfer of the oestrogens from cow's milk to formulated baby milk powder would be an additional concern, though one published study (Hamon *et al.* 1990) failed to detect such transfer.

(iii) There is now good evidence that oestrogenic activity in sewage effluent is able to exert biological effects on fish living in rivers in the UK, and elsewhere in Europe and in the USA (Purdom *et al.* 1994, Folmar *et al.* 1997). Although it was initially thought that this resulted from exposure to oestrogenic pollutants such as alkylphenols (Jobling & Sumpter 1993, see above), the most recent evidence suggests that the oestrogenicity results from oestradiol, oestrone and in some instances ethinyl oestradiol (Environment Agency 1996). It is presumed that these steroids derive from conjugated steroids excreted in human urine (mainly from pregnant women) which are deconjugated in sewage sludge via bacterial action. As sewage effluent contributes 50-90% to the flow of many European rivers and drinking water is abstracted from many of these, low level exposure of humans to these 'recycled' steroids may occur. Studies in southern Germany in 1977 reported levels of oestradiol of 0.1-0.4 pg/ml and 0.9-3.2 pg/ml of ethinyl oestradiol in drinking water obtained from wells and springs (Rurainski *et al.* 1977), and considerably higher levels of oestrogen (6-50 pg/ml) have been reported in groundwater in Israel (Shore *et al.* 1993). As ethinyl oestradiol can exert biological effects in animals *in vivo* at concentrations of ~1 pg/ml (e.g. Sheahan *et al.* 1994), the possibility of effects in humans cannot be dismissed. In the early 1960s when high (oestrogen) dose oral contraceptive pills were in use and treatment of drinking water was less developed, low level exposure to recycled oestrogens (Fig. 3) could have been of significance in the UK. However, based on the levels of these steroids reported in sewage effluent and river water in the UK (MAFF 1997), and the growing use of carbon and other filters for drinking water (which should remove these steroids) it seems unlikely that this is

a major route of human exposure today in countries with modern drinking water treatment facilities.

(iv) Oestrogens appear to have beneficial effects on skin and 'oestrogens' in one form or another might therefore have been added to certain cosmetics or be present in plant products used as ingredients in cosmetics; alternatively, one or more man-made chemicals identified (or not yet identified) as being oestrogenic (see above) may be used as an ingredient. This possible route of human exposure to 'oestrogens' has not really been explored, but considering the substantial growth in use of cosmetics in Western societies in the past century and the ease with which many chemicals applied to the skin are absorbed, it is deserving of investigation. There are isolated reports in the literature which document oestrogenic effects (usually clinical gynaecomastia) of shampoos (Edidin & Levitsky 1982, Gottswinter *et al.* 1984) or embalming ointments (Finkelstein *et al.* 1988), but it is likely that more widespread, but subclinical, effects would go undetected.

Interactive, additive, synergistic or other effects of environmental oestrogens

In reality, humans are exposed to a cocktail of environmental chemicals during their lifetime, which raises important questions about the possible additive or synergistic effects of oestrogenic chemicals. This possibility was fuelled by the report (Arnold *et al.* 1996) that two very weak environmental oestrogens (dieldrin and endosulfan) could induce a 1000-fold greater oestrogenic effect in combination than on their own, in an *in vitro* screening system based on yeast transfected with ERα. A number of other laboratories have subsequently been unable to confirm this synergism (Ashby *et al.* 1997, Ramamoorthy *et al.* 1997) and the original findings have recently been retracted by the authors (McLachlan 1997). However, the possibility of additive effects remains, as at least *in vitro* additive effects of various environmental oestrogens are clearly demonstrable (Soto *et al.* 1994, Jobling *et al.* 1995, Sumpter & Jobling 1995). What is rather more surprising is that in the presence of submaximally effective doses of oestradiol, weak environmental oestrogens, including some phthalates (Harris *et al.* 1997) and alkylphenols (Jobling *et al.* 1995, Sumpter & Jobling 1995) can still induce additive oestrogenic effects. This is the opposite of what was predicted, as it is generally considered that in the presence of a strong agonist, weaker agonists tend to have antagonistic effects.

Whether such additive effects can occur *in vivo* in man is unknown, but if this were the case it would mean that the burden of human exposure to all environmental oestrogens was key in determining risk. Unpublished data indicate that additive effects of an oestrogenic alkylphenol can still occur in female fish with high endogenous levels of circulating oestradiol (J P Sumpter, personal communication). Such effects are difficult to explain in terms of the oestrogenic potency of the chemical in question. There are several other examples which reinforce this thinking. Recent data show effects of low doses of nonylphenol (an oestrogenic alkylphenol) and bisphenol-A *in vivo* in rats on cell cycle kinetics in the mammary gland, effects which are interpreted as predisposing towards genetic instability/cell transformation etc. (Colerangle & Roy 1996, 1997); however, the authors concluded that these effects could not be explained by the oestrogenic potency of the test compounds. There are similar findings for the effects of bisphenol-A on prostate size in the mouse (Nagel *et al.* 1997) and for octylphenol on testicular size in rats (Sharpe 1995), where the effects reported are difficult to reconcile with the known oestrogenic potencies of the compounds *in vitro*. Such findings should remind us that these chemicals were not developed because of their oestrogenicity (which was largely unsuspected) but because of other, specific properties. For example, octylphenol is a surface-active agent and has been shown to be toxic to splenocytes *in vitro* at concentrations approaching 10^{-12} M (Nair-Menon *et al.* 1996). It is possible that such properties are more important, or at least an additional concern, when considering the risk to man.

Other possibilities should also be kept in mind. A second ER (ERβ) has been recently discovered which has a different and much wider tissue distribution to that of ERα, and is expressed at high levels in the prostate, endometrium and ovary and at lower levels in the testis, though possibly not in the breast (Kuiper *et al.* 1996, 1997). Compared with ERα, ERβ appears to have a somewhat different (higher) affinity for certain environmental oestrogens (Kuiper *et al.* 1997), raising the possibility that effects of the latter mediated via ERβ will turn out to be of more significance than those mediated via ERα. A further theoretical possibility is that as oestrogen-induced gene transcription involves dimerisation of two oestrogen-receptor complexes, occupancy of one of these receptor complexes by, for example an alkylphenol, might alter the conformation of the receptor dimer in such a way that transcriptional activity was enhanced (or reduced); heterodimerisation of hormone-receptor complexes of ERα and ERβ could also be of significance in this respect. Finally, in the context of cancer induction, it is theoretically possible that the close approximation of an oestrogenic chemical to DNA, when it is bound to the ER, could increase the chances of DNA damage.

To date most research effort into environmental oestrogens has concentrated on assessing their oestrogenic potency compared with oestradiol, but there are other important considerations. For example, a recent study has

shown that several environmental oestrogens described above are able to compete with physiological (endogenous) ligands for binding to SHBG and/or to the closely related androgen-binding protein, raising the possibility of a further mechanism by which hormonal homeostasis could be disturbed by exposure to these compounds (Danzo 1997).

Evidence for a role of environmental oestrogens in cancer

If it has been difficult to prove that natural hormones influence the development of cancer, then the proof that environmental chemicals are associated with the natural history of cancer can be expected to be even more tortuous because of (i) the inherently lower potency of these agents, (ii) their varied nature, and doubts surrounding which of them might be most influential, (iii) the possibility that cumulative and combined effects might be crucial - and currently we do not have accurate measures for these, and (iv) the uncertainty surrounding the biological activity of environmentals which could vary according to circumstances and differ between organs - this is likely to complicate hypothesis testing. Nevertheless, evidence has been sought and comes in two major forms: (i) epidemiological studies, which generally consist of investigations of either individuals exposed incidentally or occupationally to particular classes of environmental agents or the measurement of agents in individuals with cancer and appropriate controls, and (ii) experimental studies in which either animals are exposed to environmental agents with the objective of changing the incidence or latency of tumour development, or there is monitoring of the effects of chemicals in *in vitro* test systems, most notably cancer cell lines, to determine influences on proliferation, cellular damage or markers of hormonal action.

Epidemiological evidence linking environmental oestrogens to human cancers

Chlorinated chemicals (organochlorine pesticides/ PCBs/dioxins)

Perhaps because individual compounds may have differing biological activities, data linking organochlorines to cancer risk are confusing. In terms of individual compounds, studies on DDT exposure have been extensive but are still inconclusive (Ahlborg *et al.* 1995, Wolff 1995, Wolff *et al.* 1996). However, few of these studies had adequate exposure assessment and most did not start out specifically to investigate DDT and cancer risk. There is also a lack of consistency between investigations. For example, one cohort-based case-control study in which blood was obtained 1 to 6 months prior to the diagnosis of breast cancer indicated levels of DDE were significantly higher amongst breast cancer cases than in matched controls (Wolff *et al.* 1993). However, a more recent investigation in which blood was collected many years prior to cancer diagnosis in Caucasian, African, American and Asian women failed to find significant differences in DDE between breast cancer cases and controls, although marked differences in exposure were observed with respect to ethnicity (Kreiger *et al.* 1994). Similarly, a very recent cohort study could not demonstrate that circulating levels of DDT or PCBs were related to increased risk of breast cancer (Hunter *et al.* 1997).

It may be relevant, however, that data appear more convincing when measurements in breast tissue are considered (see also Fig. 4). For example, DDE levels appear to be approximately 50% higher in breast lipids from breast cancer patients compared with controls; this translated into a relative risk of approximately three for women with the highest tertile of exposure (Falck *et al.* 1992). A relative risk of approximately nine was reported among Canadian patients who had both high DDE levels and ER-positive tumours compared with patients with ER-negative tumours (Dewailly *et al.* 1994*a*). This association with tumour ER status has been confirmed (Pujol *et al.* 1994) and is of some interest given the oestrogenic activity of DDT and some of its metabolites and the rising rates of ER-positive cancers amongst older women (Dewailly *et al.* 1997). These findings may be more relevant because tissue levels may reflect cumulative exposure (Wolff *et al.* 1996) on account of the organochlorine compounds being sequestered within adipose tissue with a biological half-life of many years (Ahlborg *et al.* 1995).

It is thus worth noting that there are studies which have measured other lipophilic environmental chemical contaminants within normal and cancerous breast tissue. These include: (i) the finding of elevated levels of β-hexachlorocyclohexane, a lindane-related residue in women with increased relative risks for breast cancer (although no association was found for other organochlorines including DDE) (Mussalo-Rauhamaa *et al.* 1990), (ii) increased breast cancer risk associated with exposure to PCBs as assessed by the total level of PCBs in mammary adipose tissue (Falck *et al.* 1992), and (iii) higher levels of actachlorodibenzo-*p*-dioxin in samples of breast fat from patients with breast cancer compared with controls (Safe *et al.* 1991, Hardell *et al.* 1996) (although this compound is an anti-oestrogen in animals and presumably therefore should reduce risk of breast cancer) (White & Gasiewica 1993).

Limited data from women exposed to TCDD following a chemical explosion in Seveso, Italy, suggest that short-term exposures may show a protective effect (Bertazzi *et al.* 1993) (in contrast long-term exposures to

TCDD and other organochlorines used in manufacturing processes show slightly elevated risks for breast cancer (Manz *et al.* 1991, Huff *et al.* 1994)). Whilst these results are interesting, they cannot be regarded as definitive.

A complicating issue in these studies is that other risk factors may have to be taken into account. For example the organochlorine body burden in young women differs markedly according to their breastfeeding history (Dewailly *et al.* 1994*b*). Thus the apparent reduction in breast cancer incidence in women with low organochlorines could be a secondary effect of parity (see Fig. 4). Conversely, the apparent protective effect of breastfeeding on breast cancer could be mediated by increased excretion and therefore decreased exposure to carcinogenic organochlorines. The dramatic change which has occurred in the patterns of breastfeeding in many Western countries in the past half-century is also a potentially important aetiological factor in this context. Furthermore, the relative risk of DDT exposure may be transferred to the following generation if developing foetal reproductive tissues are exposed, as has been proposed by vom Saal (1995) or if contaminants are transferred from the breast to infants via the mother's milk.

Phytoestrogens

It has been argued (see Messina *et al.* 1994, Adlercreutz 1995, Clarkson *et al.* 1995, Knight & Eden 1996) that the lower incidence of breast cancer in oriental societies consuming a diet rich in soy-derived isoflavonoids (see Fig. 1) is a consequence of increased menstrual cycle length, which, over the reproductive lifespan of a woman, equates to reduced exposure to endogenous oestrogens and thus reduced risk of developing breast cancer. The demonstration by Cassidy *et al.* (1994) that Western women who are placed on a soy diet have a longer menstrual cycle is consistent with this thinking. Three case-control studies have reported protective effects of soya bean products against the development of breast cancer (Nomura *et al.* 1978, Hirayama 1986, Lee *et al.* 1991) but two recent investigations in China have failed to confirm these findings (Hirohata *et al.* 1985, Yuan *et al.* 1995). Arguments similar to those for breast cancer have been voiced regarding the low incidence of prostate cancer in Oriental societies (Messina *et al.* 1994, Adlercreutz 1995) (see Fig. 1), though the biological basis for such claims is less well substantiated.

There is also insufficient epidemiological evidence to suggest that the agonist properties of the isoflavonoids may cause an increased risk of endometrial carcinoma (Miyazawa 1976).

Although it is obviously difficult to prove cause and effect in situations in which many fundamental differences exist in the diet (e.g. in fat content of Oriental and Western diets), the available data from a wide range of *in vivo* and *in vitro* studies (see below) suggest quite strongly that consumption of soy and soy-derived isoflavonoids probably does confer some protection against cancers, though whether this is solely the result of their oestrogenicity or is a consequence of other biological properties is far less clear (Messina *et al.* 1994, Adlercreutz 1995, Knight & Eden 1996).

Experimental evidence linking environmental oestrogens to human cancers

Chlorinated chemicals (organochlorine pesticides/ PCBs/dioxins)

Two major metabolites of DDT (DDE and TDE (bis(4-chlorophenyl)-2,2-dichloroethane)) are known to be carcinogenic in animals - DDE induces liver tumours and TDE causes liver, lung and thyroid gland tumours (Huff *et al.* 1996). However, DDT has not been shown to induce tumours of the mammary gland (Wolff *et al.* 1996). Indeed, certain PCB congeners, as well as TCDD possess anti-oestrogenic properties that may protect against breast cancer (Safe *et al.* 1991). Furthermore, TCDD administered to rats reduced the number of spontaneous mammary gland and uterine tumours and diminished the size of chemically induced mammary tumours (Kociba *et al.* 1978). In contrast *o,p′*-DDT mimics oestradiol stimulation of breast cancer cells, causing them to enter the cell cycle by affecting key regulator elements such as cyclin D1, Cdk2 activation and phosphorylation of the retinoblastoma protein (Dees *et al.* 1997) though *o,p′*-DDT is some 100-300 times less potent than oestradiol in this respect. The steroidal anti-oestrogen ICI 182 780 prevents both growth and Cdk2 activation induced by oestradiol or *o,p′*-DDT. Consistent with these findings, such effects are not elicited by *o,p′*-DDT or oestradiol in ER-negative HS578Bst breast cancer cells or in rat liver epithelial cells. These data strongly suggest that the influences of *o,p′*-DDT in this context are mediated via classical oestrogenic actions and effects on other signalling systems are not seen. In contrast DDT at low concentrations (i.e. 10 nM) stimulates c-erbB2, c-met growth factor receptor, STATs (signal transduction-activating transcription factors) signal transduction processes and proliferation of breast epithelial cells which are ER-negative (Shen & Novak 1997). These results provide evidence of multiple signalling mechanisms by which environmental chemicals may stimulate cell proliferation and/or tumorigenesis and thereby function as xenomitogens. Since receptor tyrosine kinase and JAKs (Janus activation kinases)-STATs pathways are key regulators of cell proliferation and differentiation in biological systems it has been suggested that the interaction of environmental chemicals with these signalling pathways will affect cell growth and

differentiation, and ultimately, influence pathogenesis, especially if such chemicals persist over several decades.

Bisphenol-A, alkyphenols and phthalates

As awareness of the oestrogenicity of these compounds is very recent, experimental data which assess their possible role in cancer induction are only just beginning to emerge.

Nagel *et al.* (1997) have shown that exposure of male mice to bisphenol-A *in utero*, at levels likely to approximate some human exposures, results in increased prostate size in adulthood; similar effects were obtained by elevating endogenous levels of oestradiol by 50% or by the administration of very low or high doses of DES, whereas doses intermediate between these two extremes were without significant effect (vom Saal *et al.* 1997). This U-shaped dose-response is difficult to interpret but, at face value, it suggests that extremely low levels of bisphenol-A, or presumably any other oestrogen, could exert biological effects on the prostate during early development. Another recent study in Noble rats demonstrated that administration of doses of bisphenol-A as low as 100 µg/kg per day exerted major effects on lobular maturation of mammary gland ducts and altered cell cycle kinetics (Colerangle & Roy 1997), findings similar to those found by the same authors involving administration of low doses of nonylphenol (Colerangle & Roy 1996). In both of the latter studies, the authors concluded that the effects of bisphenol-A and nonylphenol could not be explained by their oestrogenicity, because of the low doses involved. However, this conclusion is opposite to that of Nagel *et al.* (1997), regarding the effects of bisphenol-A on the prostate, as they concluded that bisphenol-A did not bind to serum proteins, which thus increased its oestrogenicity *in vivo* relative to oestradiol. Curiously, Nagel *et al.* (1997) concluded that nonylphenol did bind to serum proteins and was consequently less oestrogenically active *in vivo*, a finding which contrasts completely with the effects of low doses of nonylphenol on the mammary gland reported by Colerangle & Roy (1996). Irrespective of these inconsistencies, the data suggest that weak oestrogens such as bisphenol-A and nonylphenol could be of potential significance in the aetiology of human prostate and breast cancer, though whether this is because of their oestrogenicity or because of other chemical properties remains to be resolved.

Another recent study (Singletary *et al.* 1997) has shown that high doses of BBP (100-500 mg/kg) can inhibit mammary gland DNA adduct formation and tumorigenesis induced by dimethylbenz(a)anthracene in rats. Although the mechanism behind this effect is unclear, the recent discovery that BBP, and the related compound DBP, are also anti-androgenic (see above) at these concentrations may be relevant.

Phytoestrogens

A high-soy diet, as well as its major phytoestrogen genistein, can reduce both the incidence and growth of carcinogen-induced tumours in experimental animals. This is particularly so for mammary tumours (Troll *et al.* 1980, Barnes *et al.* 1990, Hawrylewicz *et al.* 1991). Confirmation that these effects of soy are caused by isoflavones is suggested by the observations that soya from which isoflavones have been chemically removed does not influence mammary carcinogenesis (Barnes *et al.* 1990). In these model systems, tumour formation is negatively correlated with total dietary isoflavone concentration. Interestingly, administration of genistein to rats in the perinatal period increases the latency period for appearance of mammary tumours (Lamartiniere *et al.* 1995).

Diphenolic lignans and isoflavonoids have been suggested to be anti-oestrogenic because of their ability to (i) antagonise oestrogen binding to receptors (Zava & Duwe 1997), (ii) increase levels of SHBG (Adlercreutz *et al.* 1992), and (iii) inhibit *in vitro* growth of hormone-sensitive cancer cell lines over concentration ranges which may be achievable *in vivo* (10 nM-20 µM) (Zava & Duwe 1997). However, other research suggests that isoflavonoids may have oestrogen agonist effects - indeed even in the same assay systems some flavonoids may be oestrogen agonists whilst others are not. Similar divergence in effects on cell growth may occur (Peterson & Barnes 1991), although generally growth stimulation of ER-positive cells by isoflavones closely parallels binding affinity to ERs (Zava & Duwe 1997). However, the same isoflavone may also exert different effects depending on concentration. For example, at low physiologically relevant concentrations (1 µM-1 mM), genistein behaves as a pure oestrogen agonist, inducing the oestrogen-regulated gene-product, pS2, and stimulating cell proliferation. In contrast, higher concentrations of genistein (≥10 mM) are growth inhibitory and reduce proliferation in both ER-positive and ER-negative breast cancer cells (oestradiol does not reverse the effect) (Peterson & Barnes 1991). This and other *in vitro* studies mean that serious consideration needs to be given to the possibility that isoflavones, specifically genistein, do not inhibit cell growth or prevent cancer by classical anti-oestrogenic mechanisms but potentially via a variety of other cellular mechanisms.

In this respect, genistein has been shown to (i) inhibit activity of the epidermal growth factor receptor tyrosine kinase (Clark *et al.* 1996), (ii) influence *in vitro* angiogenesis and endothelial cell proliferation (Fotsis *et al.* 1993) - although the effects on angiogenesis require concentrations an order of magnitude higher than the levels required to inhibit cancer cell growth, (iii) block the activity of topoisomerase II (Okura *et al.* 1988, Markovitz

et al. 1989, Constantinou *et al.* 1990), although again at IC_{50} values for growth inhibition, little DNA damage is observed, (iv) have an anti-oxidant effect by decreasing the production of reactive oxygen species by tumour cells and those of the immune system (Tanimura *et al.* 1992, Utsumi *et al.* 1992, Wei *et al.* 1993), (v) regulate the synthesis and activity of phase I and II enzymes such as cytochrome p450 cyp 1A1 and catechol-*O*-methyl transferase and glutathione transferase (all of which may be implicated in mechanisms of malignant growth) (Zhu *et al.* 1994), and (vi) inhibit aromatase and type 1 17β-hydroxysteroid oxidoreductase enzymes (Wang *et al.* 1994, Adlercreutz *et al.* 1995, Makela *et al.* 1995) which are responsible for producing active oestrogens. Faced with this impressive armoury of biological properties, one word of caution. Most culture experiments, which have generated these data, have been performed with established human cancer cell lines and do not address the role of phytoestrogens in normal cell proliferation.

Concluding remarks

Although we emphasise that there are no data which unequivocally link hormone-dependent cancers with exposure of man to 'environmental oestrogens' or other hormonally active chemicals (i.e. anti-androgens etc.), it also should be now apparent to the reader that a surprising number of known hormonally active chemicals are present in our modern environment (let alone those which we do not know of but suspect are there). It is also clear that some of these chemicals do cause disorders (usually of reproduction) in a range of animals, especially aquatic life (see Toppari *et al.* 1996). Faced with this evidence it would seem both foolish and dangerous for us to conclude that man is not affected by such chemicals because we have no proof of this. Man does not live in rivers or in the sea, so we are presumably less at risk from the suspect chemicals which accumulate in the aquatic environment, but arguably this is counterbalanced by the fact that we are at the top of the food chain and thus are particularly at risk from lipophilic, bioaccumulative chemicals. The fact that the incidence of cancers of the breast and prostate are also clearly influenced by dietary factors, in particular the consumption of animals fats (Armstrong & Doll 1975, Nomura & Kolonel 1991, Cassidy *et al.* 1994, Hunter & Willett 1996), is obviously of relevance in this context.

Both our exposure to hormonally active, environmental chemicals and development of cancers of the reproductive system are chronic rather than acute processes. If there is any causal relationship between these lifelong events it will obviously prove extremely difficult to establish. It is pertinent therefore to ask what additional information is required to make the case compelling? It is essential to define the underlying cause(s) of reproductive cancers and this will surely come with greater understanding of the basic biology of carcinogenesis and tumour growth. Secondly, it will be important to learn more about the biological effects of environmental chemicals in organs susceptible to cancers and on the behaviour of cancers growing in these sites. In this respect, consideration will have to be given to end-point measurements. Is it sufficient to assay oestrogenic potential or should there be a battery of tests which would include measurements of cellular proliferation and DNA damage? The present emphasis of research is very much on the former, but hormonal influences may only be incidental to carcinogenesis, their effect being attributable to stimulation of cell proliferation and DNA damage. A high rate of cell proliferation is recognised as predisposing towards greater cancer risk in the long term (Preston-Martin *et al.* 1990, Iversen 1992). However, even if environmental oestrogens potentially contribute to cancer development because of their oestrogenicity and the resultant effect on cell proliferation, it must not be forgotten that the production of endogenous oestrogens, which physiologically regulate cell proliferation in reproductive tissues, is homeostatically controlled by a series of feedback loops. Any intake of exogenous oestrogens may therefore be compensated for by reduced production of endogenous oestrogens, though complicating factors such as binding to SHBG, bioaccumulation, age at exposure etc. have to be taken into consideration (Fig. 5).

If and when it is proven that specific environmental chemicals are responsible for cancer, what can we do about it? It may be possible (i) to reduce general exposure by restricting production or sale of synthetic agents, and (ii) to target specific risk groups (although they may be difficult to define). If the problem lies in the food chain or in lifestyle (e.g. cosmetics usage), it will be necessary to consider changing dietary and other habits through social pressure. However, if the history of the knowledge that cigarette smoking causes cancers is taken as an example, corrective measurements may not be easy to implement, especially if there is a long latency period between exposure and event (as seems likely). Younger people may be resistant to change if they cannot see immediate benefits. Pregnant women may be more motivated to protect their unborn children if it can be proven that perinatal exposure is critical but pregnancy itself may complicate intervention. Finally, one could consider therapeutic interventions. Potent specific anti-hormone therapies have been recently developed which are powerful tools (Howell 1996, Miller 1997) but the long-term administration of such agents may be impracticable, at least until the side-effects and toxicity of such therapies are known. The knowledge accumulated from the

Figure 5 Diagrammatic summary of the major changes over the past 50 or so years which have altered the exposure of the human female to oestrogens, whether produced endogenously or derived from the environment. Although there have been numerous changes which are likely to have increased lifetime exposure to oestrogens (right of diagram), these may be counterbalanced by altered endogenous hormone production, better nutrition or by the ingestion of oestrogen antagonists. There are arguments for phytoestrogens being viewed as either oestrogen agonists or antagonists, so it is uncertain as to which side of the diagram they should be placed. Changes which are likely to have significantly altered human exposure to environmental hormonally active compounds are indicated by arrowheads. Note that the relative size of each section is determined by practical convenience and is not indicative of the relative importance or contribution of this factor, as this is largely unknown.

treatment of patients with cancer in whom such agents are currently being tested will be invaluable.

Should we invest more research monies in environmental oestrogens, bearing in mind that other research efforts may suffer as a consequence? Perhaps the most persuasive argument is that there are very likely to be significant health benefits if the research effort is conducted 'with an open mind'. It is becoming increasingly clear that, in both males and females, oestrogens play a role in cardiovascular disease and blood lipid chemistry, in bone growth and remodelling, let alone their role in cancers of the reproductive system. Some of our exposure to environmental oestrogens is likely to be beneficial. This being the case, we have nothing to lose and everything to gain from improved understanding of the sources and actions of environmental oestrogens on the body and its consequences. However, we must realistically expect little immediate impact from such research. There are many practical problems in providing proof of principle - it will be necessary to perform studies in individuals at risk or in patients with cancer. What assays will be performed in these populations and in what tissue? Do we have, or can we develop, reliable relatively non-invasive assays for cumulative chemical exposure which will yield evidence of previous exposure? Given that influences on cancer are likely to be multivariate, how do we control for other factors, especially when there are variable estimates as to the contribution which environmental factors make to cancer incidence and how this interacts with inherited genetics.

The mere fact that some or all of reproductive cancers have increased in incidence at the same time as the release to our environment of many man-made chemicals with intrinsic oestrogenic, anti-oestrogenic or anti-androgenic activity, is a coincidence that would surely have given Sherlock Holmes pause for thought. In trying to establish whether or not there is any causal relationship between these two events, we should perhaps adopt two other guiding principles from Sherlock Holmes. First, the truth will only emerge from detailed (scientific) detective work, not from emotion or preconviction. Secondly, eliminate what is not possible and what is left is the truth. If exposure to environmental, hormonally active chemicals plays any part in the aetiology of human reproductive cancers, the effect is likely to be insidious and difficult to prove. It is probable therefore, that only by elimination of impossibilities and by then 'weighing' the remaining evidence, will we be able to establish the likelihood of whether environmental, hormonally active chemicals contribute to human cancer risk.

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