

Review

Endocrine disruptors: A human risk?

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Abstract

Endocrine disruptors (EDs) alter normal hormonal regulation and may be naturally occurring or environmental contaminants. Classically, EDs act genomically, with agonistic or antagonistic effects on steroid receptors and may alter reproductive function and/or cause feminisation by binding to oestrogen or androgen receptors; their binding to the thyroid receptor may dysregulate the neuroendocrine system. Recently, it has been shown that EDs can also act by non-genomic mechanisms, altering steroid synthesis (inhibition of cytochrome P450 isoforms) or steroid metabolism. The alkylphenol and phthalate plasticisers inhibit the inactivation of oestrogens by sulphation (via SULT 1A1 and 1E1 isoforms) and so cause a rise in levels of the free active endogenous oestrogens. A range of ED effects have been shown in mammals, fish, birds, reptiles, amphibia and aquatic invertebrates but it is not yet clear whether these processes also occur in human beings. It is evident that EDs, as well as altering reproduction, can cause changes in neurosteroid levels and so have the potential to affect immune function, behaviour and memory. This may be of long-term concern since traces of EDs such as plasticisers, brominated fire retardants, sunscreen agents and cosmetic ingredients are widely distributed in the environment and in human biofluids.

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1. Introduction

Endocrine disruptors (EDs) are compounds that alter normal hormone regulation. They have been defined by the European Commission as ‘an exogenous substance or mixture, that alters function(s) of the endocrine system and consequently causes

adverse health effects in an intact organism or its progeny or (sub)population’. They may be naturally occurring, such as the antioxidant flavonoids, which are found in fruits and vegetables, or may be industrial chemicals, such as some types of plasticiser, which act as environmental contaminants. A wide range of species, from crustaceans, fish, birds through to mammals and man, have been reported as being dysregulated by EDs although their significance to human beings is still unclear.

The biological actions of hormones, including oestrogens, androgens, progesterone, thyroxine and the neurosteroids

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pregnenolone and dehydroepiandrosterone (DHEA), are mediated via high affinity protein receptors within the target cells. Steroids such as oestrogens normally circulate as their sulphated derivatives, which have no hormonal effects; the free steroid is released at the target tissue by the action of tissue-bound sulphatase enzymes. Other steroids circulate the blood stream bound to carrier proteins or to serum albumin. All steroids are fat-soluble and readily cross the cell membrane, interacting with dimeric receptor proteins; in the case of oestrogens these are ER- α and ER- β , although recent evidence suggests a third class, putative-ER or ER- γ , is involved in fish and possibly mammals (Dodge et al., 1996; McLachlan et al., 2001). The affinity of a steroid for its receptor is so great that it is the equivalent of being able to taste a teaspoonful of sugar dissolved in the water of a swimming pool. The steroid-receptor complex binds to target regions of DNA termed “response elements”. This activates the cascade of reactions, which are the response to the presence of steroids. Classically, EDs have been viewed as exerting their effects exclusively by genomic mechanisms, acting as steroid agonists by binding to the receptor. However, there is evidence that some oestrogenic compounds do not act via the oestrogen receptors and hence non-genomic effects may also play a part in the mechanisms of action of EDs. This possibility means that a range of test methods must be developed to predict ED potential in commercially available compounds, because many biochemical pathways may be targets.

2. Genomic mechanisms of ED action

Classical genomic effects have been demonstrated for natural compounds (e.g. coumestrol), pharmaceuticals (e.g. tamoxifen and diethylstilbestrol) and industrial chemicals (e.g. octylphenol and bisphenol-A). These compounds all bind to oestrogen receptors and so act as pseudoestrogens *in vivo*, giving feminising effects (Gray et al., 1999; Laws et al., 1995; Stroheker et al., 2004). Feminisation can also occur indirectly—the fungicide vinclozolin binds competitively to the androgen receptor (Shono et al., 2004), blocking the cellular actions of testosterone on androgen-dependent tissue growth and behaviour patterns. Other compounds, such as chlordecone, inhibit binding to the oestrogen and progesterone receptors (Guzelia, 1982), whereas bisphenol-A can block ligand binding to the thyroid receptor (Moriyama et al., 2002). As there is ‘cross-talk’ between the oestrogen and thyroid receptors, compounds which are oestrogen-receptor agonists may also affect the neuroendocrine development which is regulated by thyroid hormones. These effects are not yet fully characterised or understood but appear to involve modulation of neuronal patterning with potential long-term effects.

3. Non-genomic mechanisms of ED action

When receptor-binding activities of some EDs are compared to their biological effects, it is clear that their activity is not exclusively due to their roles as steroid agonists. As well as acting as exogenous steroid mimics, EDs may also exert effects by

altering the synthesis or availability of endogenous hormones. Steroids are initially formed from cholesterol via a series of reactions, which can involve the cytochrome P450 (CYP) isoforms. CYPs 2C11, 2A1, 2B1, 3A1 and 2C19 catalyse critical stages; CYP 2C19 is the enzyme ‘aromatase’ which controls the formation of oestrogens (which contain an aromatic ring) from precursors. Expression of CYPs from the 2B and 2C families occurs during the earliest stages of foetal development (Li You, 2004) and, because they can be induced by environmental contaminants such as DDT and its analogues, this gives another mechanism by which ED activity can take place. Compounds of the azole type, such as ketoconazole and the fungicide fenarimol, inhibit these CYP isoforms and consequently can also affect steroid synthesis (Hirsch et al., 1987) while the now-banned anti-fouling agent tributyltin and its metabolites, which have strong ED potential, are thought to act by the same mechanism, probably by inhibition of aromatase (Alzieu, 2000).

Oestrogens and their steroid precursor, DHEA, are normally transported in the bloodstream as their sulphonate esters and, as such, do not enter the target cells. The esters are synthesised, using the cofactor 3'-phosphoadenosine-5'-phosphosulphate, by cytosolic sulphotransferase enzymes with the isoforms SULT 1E1 and SULT 2A1 having particular affinities for oestrogens and DHEA, respectively. Sulphatases located on the surfaces of the target cells release the free steroids, which can then enter the cell. Any excess steroids are re-conjugated and excreted from the cells; the importance of this pathway has been shown by inserting the gene for SULT 1E1 into the MCF-7 breast cancer cell line resulting in a decrease of the cells' proliferative response towards oestradiol (Falany et al., 2002). Hence, compounds which alter the sulphotransferase/sulphatase activity ratio can potentially affect the availability of endogenous oestrogens to target tissues (Kirk et al., 2001). Many phenolic compounds, including the alkylphenol plasticisers which are known EDs, inhibit both SULT 1E1 and SULT 2A1, and also act as substrates (and thereby competitively inhibit the sulphonation of other compounds) for the phenolsulphotransferase SULT 1A1. Although this isoform mainly sulphonates simple phenols, it will accept oestrogens as substrates at relatively high concentrations and we have found that it will readily sulphonate alkylphenols at sub-micromolar levels (Harris et al., 2005). However, it should be remembered that while alkylphenols can cause significant inhibition of sulphotransferase activity at sub-micromolar concentrations, there are naturally occurring compounds, e.g. some types of flavonoid, which are more potent by two or more orders of magnitude (Kirk et al., 2001). In addition, while the inhibition of sulphotransferase activity could cause a localised increase in free oestrogen concentration, it may also bring about a general, and perhaps compensatory, decrease in the availability of oestrogens by reducing the formation of the transport conjugate. The hydroxylated metabolites of polychlorinated biphenyls (PCBs) are potent competitive inhibitors of thyroxine binding to the human thyroid hormone transport protein, transthyretin, and also inhibit SULT 1E1 so that they act as EDs by at least two different mechanisms (Miyazaki et al., 2004).

4. Effects of EDs in mammals

In mammals, including man, the steroidal sex hormones (androgens and oestrogens) regulate foetal developmental processes such as differentiation and sex determination. Androgens, including testosterone, regulate the development of a male phenotype and disruption of steroid levels at critical stages can cause feminisation of male foetuses if there is an excess of oestrogenic compounds or a deficiency of androgens. Conversely, female foetuses can be masculinised by androgen excess or oestrogen deficiency at specific time points in foetal differentiation.

Studies in mammals have shown that reproductive function can be affected by compounds of both natural and industrial origin. Phytoestrogens are produced by plants, possibly as protectants against attack, and fall into three main categories, flavones/isoflavones, coumestans and lignans (Dodge, 1998). Phytoestrogens from clover have long been recognised as the cause of reproductive dysfunction and infertility in sheep and the compounds responsible have been identified as equol and also coumestrol (Rossiter and Beck, 1966) which binds as strongly as 17- β -oestradiol to both human ERs (Morito et al., 2002). It is interesting that the neurobehavioural effects of coumestrol are anti-oestrogenic in rodents so that the results of a phytoestrogen diet may be complex (Whitten et al., 2002). Industrial chemicals can also act as EDs in mammals. Phthalate esters such as diethylhexylphthalate (DEHP) are a major component in the polyvinyl chloride (PVC) plastics, which are frequently used in medical tubing and blood storage bags. DEHP is readily released from medical devices, and it and its metabolites have been found in urine from premature babies on intravenous infusions in a neonatal intensive care unit (Calafat et al., 2004). DEHP is an endocrine disrupter in rats, producing Leydig cell hyperplasia and affecting systemic physiology as well (Akingbemi et al., 2004). The main metabolite of DEHP, monoethylhexylphthalate (MEHP) acts as an anti-oestrogen in rats at low doses but has oestrogenic activity at higher levels (Shono and Suita, 2003). Di-*n*-butylphthalate (DBP) similarly reduced fertility in rabbits although there appeared to be a 'critical window' of dosing for effects to become apparent and the intrauterine period was the most sensitive (Higuchi et al., 2003). DBP binds relatively weakly to the human oestrogen receptor and probably acts via anti-androgenic or by non-genomic mechanisms. Other plasticisers, such as nonyl and octyl phenols and their derivatives also bind to oestrogen receptors. Bisphenol-A (BPA) similarly acts at oestrogen receptors in reproductive tissues and is an agonist for CNS receptors; in high doses it causes reproductive toxicity in rats and mice (Tan et al., 2003). Some UV filters (found in sunscreen creams) are also oestrogenic; 4-methylbenzylidene camphor and 3-methylbenzylidene camphor are ligands for both ER subtypes, particularly ER- β , and have shown oestrogenic effects *in vivo* in rats (Schlumpf et al., 2004).

5. ED action in fish

Freshwater fish in particular have been the subject of many studies into the actions of EDs in the aquatic environment. Any lipophilic contaminants in the water surrounding the fish

are readily absorbed; as the detoxication systems in fish are less effective than those in mammals, the effects of EDs are more apparent. Bioaccumulation effects also occur, especially in bottom-feeding fish and those at the top of the food chain, as most EDs are lipid-soluble and so are concentrated into the fat of the ingesting organism. Most of the work has been carried out on oestrogen exposure, using the presence of vitellogenin in the plasma of male fish as a biomarker. This protein is an egg yolk precursor, produced by oviparous female fish in response to circulating plasma oestrogens. Other bioassays include the effects of EDs on survival, reproduction and growth, although toxicity effects must be taken into account as fish are very susceptible to phenolic compounds. A variety of effects of EDs on fish populations have been reported. The effluent from a domestic waste water effluent from a sewage treatment works was shown to be oestrogenic, causing hermaphroditism in a fish population and an increase in plasma vitellogenin in male fat-head minnows (Hemming et al., 2001). It is possible that this reflects the presence of steroidal contraceptive agents such as 17- β -ethinyloestradiol, which are not easily removed by conventional sewage treatments (Cargouet et al., 2004; D'Ascenzo et al., 2003). Effects of this type have been reported for a wide range of fish species; the phenotypic results can be complex with 'intersex' fish being found but the experiments generally show that fish reproduction is very sensitive to environmental contamination, especially from sewage plants and industrial discharges (Arukwe, 2001).

Plasticisers are examples of environmental compounds, which affect fish reproduction. Alkylphenols such as nonyl and octyl phenol are weak oestrogen structural mimics, and exposure of fish to these compounds has been linked with increased mortality rates, reduced reproductive capacity and vitellogenin synthesis in male fish with yolk degeneration in piscine oocytes (White et al., 1994). Like mammals, fish also possess sulphotransferases and they are known to use sulphated steroids as pheromones. Although relatively little is known about fish sulphotransferases, if these enzymes were also to be significantly inhibited by some EDs this could result in a reduction in pheromone production with consequent effects on shoal behaviour and breeding. Levels of plasticisers in river water can be surprisingly high—bisphenol-A, which is used to make polycarbonate plastics and epoxy resins, was found in a range of samples from Germany (500 pg/l to 16 ng/l), as were 4-nonylphenol (2–15 ng/l) and 4-*tert*-octylphenol (150 pg to 1.5 ng/l). Similar analyses from Turkish rivers found that alkylphenols were not present in the water but were concentrated in the sediments (1–4.46 μ g/g) (Uguz et al., 2003). Higher values have been reported from England (15 μ g/g) and the USA (70 μ g/g) (Blackburn et al., 1999). As would be expected for these lipophilic compounds, analysis showed that alkylphenols contaminate the tissues of the fish in these waters, values of 0.1–0.8 μ g/g nonylphenol being reported for samples from both English and Turkish rivers (Blackburn et al., 1999; Uguz et al., 2003). At least in summer months, fish may also be contaminated with camphor derivatives from sun-screen creams used by people swimming in lakes and rivers (Poiger et al., 2004). The levels in fat tissue in German fish from the Meerfelder Maar

were similar to the concentration of 7 mg/kg, which has been reported to affect development and reproduction (Inui et al., 2003). Effects of EDs are not limited to lake and river fish as ocean-going fish have also been affected by exposure to these compounds. Studies in the Canadian Atlantic salmon (*Salmo salar*) have shown that 4-nonylphenol can disrupt the growth hormone/IGF-1 axis, particularly at the parr-smolt transformation stage, so that this species may be susceptible to oestrogenic run-off in rivers supporting sea salmon stocks (Arsenault et al., 2004). Fish cell lines have been developed as tools in ecotoxicology and should help to improve our understanding of the mechanisms of actions of EDs in fish (Fent, 2001) as metabolic studies have generally been hampered by a lack of in vitro models.

6. ED effects in birds

Unlike mammals, sexual differentiation in birds is dependent on oestrogen levels, which regulate production of the female phenotype. If oestrogen synthesis is blocked by injection of an inhibitor of the enzyme aromatase into the fertilised egg, then the resulting female chicks may be phenotypically sex-reversed with bilateral testicles, sperm production and male behaviour patterns. Feminised male birds with an ovotestis and reduced male copulatory behaviour patterns can be induced by EDs such as *o,p'*-DDT which are agonists at the oestrogen receptor (Fry and Toone, 1981).

Some of the earliest work on ED effects was carried out in bird populations affected by DDT and other organochlorines. These compounds are now banned over most of the world but their use in the 1960s coincided with a decrease in reproductive ability of birds, particularly those at the top of the food chain such as raptors and gulls. Egg-laying and calcification of the egg shell in birds is oestrogen and Vitamin D dependent; in a typical example, the presence of organochlorines not only resulted in feminisation of male Japanese quail chicks but also led to shells which were non-viable because they were too fragile to hatch successfully or so thick that the chicks could not emerge (Halldin et al., 2003).

7. Effects of EDs of reptiles, amphibia and aquatic invertebrates

Sex determination in reptiles has an environmental component, being partly determined, at least in alligators, by temperatures surrounding the incubating eggs. Nevertheless, ED effects have been well documented in Florida alligators, where exposure to DDT and its metabolite DDE markedly decreased the egg hatching rate and increased juvenile mortality (Vonier et al., 1996). The surviving animals showed raised oestradiol/testosterone ratios in both sexes. Contamination in Lake Apopka was also linked to disruption of bone resorption, showing that several physiological systems may be affected (Lind et al., 2004). The commercially important weed killer atrazine has oestrogenic effects on tadpoles and adult frogs, converting adult males into hermaphrodites and retarding gonadal development (Hayes et al., 2003). The effects were seen at 0.1 ppb, below the 3 ppb concentration permitted in drinking water and paral-

led those found in rats where atrazine was shown to act as an ED by directly inhibiting Leydig cell production of testosterone (Friedmann, 2002).

As invertebrates comprise about 95% of all terrestrial and aquatic animal species, they are frequently included in regulatory assessment schemes for EDs. However, laboratory data on a few species is frequently extrapolated to the wider range in nature. Chronic ecotoxicity testing for ED potential is usually carried out on crustaceans, insects, annelids and molluscs (including bivalves and gastropods) although there is growing interest in the nematode *C. elegans* as a model organism (Hutchinson, 2002). It is generally assumed that the mode of action of EDs is similar across the major evolutionary phyla and those tested so far seem to have affected some parameter of growth or reproduction in all species.

Tributyltin (TBT) was widely used in the 1970s as an anti-fouling paint on ships' hulls and has been shown to damage reproduction in molluscs, especially oysters, which are commercially important. Exposure to TBT in molluscs leads to a rise in testosterone levels in females and a partially masculinised phenotype, probably due to inhibition of aromatase (Matthiessen and Gibbs, 1998).

Amphipods such as *Gammarus pulex* respond to oestrogenic compounds in water from sewage treatment works in the same way as fish, with abnormalities of oocytes and vitellogenesis (Gross et al., 2001). Exposure to nonylphenol has been shown to affect daphnid fecundity and sex determination and also to be toxic to the copepod *Tisbe battagliai* (Bechmann, 1999). Barnacles, which cause marine biofouling, have been studied as models of endocrine development mechanisms. The plasticiser 4-*n*-nonylphenol (like oestradiol) was shown to induce a vitellin-like protein (Billinghurst et al., 2000) although the timing of exposure was critical for disruption of larval development.

Echinoderms, such as the crinoid *Antedan mediterranea*, synthesise vertebrate-type steroids, which control reproductive competence and growth. Regeneration of lost arms in these creatures is controlled by regulatory factors secreted by the nervous system and has been used to study neuroendocrine effects of ED contamination. *A. mediterranea* is benthic and so likely to be in contact with contaminated sediments. Regeneration time and morphology of the new tissue were both shown to be affected by PCBs; this may be a simple test system to detect ED effects in the CNS (Candia et al., 2001). However, results on aquatic invertebrates should be interpreted with caution since effects, which appear to be due to ED action may simply reflect general toxicity and reduced energy production (Barata et al., 2004).

8. Non-reproductive effects of EDs

Most of the research on EDs has focused on impairments to reproductive potential, probably because this aspect is emotive and also easily visible. However, recent work has raised the possibility that EDs also affect thyroid function and that they can have profound but subtle effects on behaviour and memory (Jahnke et al., 2004). Thyroid hormones such as thyroxine not only control metabolic rates but also are involved in regulation and differentiation of the developing central nervous system.

Studies on a range of EDs showed that each compound elicited its own spectrum of alterations to thyroid hormone metabolism and function (Ishihara et al., 2003) while thyroxine binding to the transport protein transthyretin is a target for plasticisers such as nonylphenol, bisphenol-A and butylbenzylphthalate (Harvey and Johnson, 2002). Flavonoids and isoflavonoids can also affect thyroid hormone metabolism in vitro but as, unlike BPA, they are relatively poorly absorbed and less fat-soluble, they may pose a reduced risk. The chlorinated pesticides such as PCBs and TCDs cause disruption of thyroid hormone homeostasis in animal studies and there is some evidence that PCBs cause developmental neurotoxicity in man (Hagmar, 2003; Winneke et al., 2002). The UV-filtering camphor derivatives, particularly 4-methylbenzylidene camphor, appear to interact with the thyroid system in both male and female rats (Poiger et al., 2004) and so may have long-term effects. EDs can also affect behaviour. Japanese quail exposed to endocrine disrupters had altered responses to environmental stimuli (Ottinger et al., 2001) and similar results have been reported for the threespined stickleback (*Gasterosteus aculeatus*), which were subject to higher levels of predation because they behaved less circumspectly. Exposure to 100 ng/l of ethinyloestradiol increased growth and promoted risky behaviour, resulting in increased mortality (Bell, 2004). Prenatal exposure to EDs has been shown to change the behavioural development of mice, altering aggression and reactivity (Palanza et al., 1999). It is not clear why this association occurs but it may be due to variations in levels of neurosteroids. Evidence has recently been obtained which shows that neurosteroid metabolism may be altered by EDs, as they can affect the metabolism of DHEA. DHEA, with pregnenolone, acts as a modulator of neuronal transmission and is found all over the central nervous system at low levels. However, the sulphated derivatives are specifically localised in the frontal cortex and hippocampus and are essential for memory formation and retention. Rats given inhibitors of SULT 2A1, the sulphotransferase isoform responsible for conversion of DHEA to DHEA-sulphate, have greatly decreased memory (Vallee et al., 2001) and it seems likely that the same effects could occur in man. Some EDs, particularly *tert*-octylphenol (Harris and Waring, 2005) and the fungicides fenarimol and prochloraz (Andersen et al., 2002) are potent inhibitors of SULT 2A1 and, as they are very fat-soluble, it is possible that a lifetime exposure to these plasticisers could impair the function of the ageing brain. There is some evidence that children whose mothers were exposed to high levels of PCBs and polychlorodibenzofurans (PCDFs) have lower IQ scores, cognitive dysfunction and memory problems, with behavioural disturbances, although the mechanisms are not known (Hsu et al., 1985). Prenatal exposure to PCBs was found to delay processing in the CNS in Dutch babies (Vreugdenhil et al., 2004) suggesting that subtle neurological damage can occur.

9. EDs and human health

Early health effects from ED contamination were noted in the 1940s when aviation crop-dusters handling DDT were found to have reduced sperm counts (Singer, 1949). Similarly, workers at factories producing chlordecone were reported to have lost

libido, become impotent and have low sperm counts (Guzelia, 1982). Both compounds are oestrogenic EDs, as has been shown in experiments with laboratory animals. These results underlie the controversial suggestion that ED contamination of the environment underlies a world-wide decline in human sperm production (Sharpe and Skakkeback, 1993). A meta-analysis of 61 studies with about 15,000 men found a decrease in sperm concentration and semen volume from 1938 to 1990 (Eertmans et al., 2003). However, there were various sources of bias. Other studies have shown a large variability in sperm quality and quantity with the geographical source of samples, and also unchanged or even increased sperm counts and seminal volume (Fish et al., 1996). Clearly, this is a complicated area. Nevertheless, it is possible, as Sharp and Skakkeback have proposed, that in utero exposure to environmental oestrogens could affect some individuals, as these effects have been reported in animals (Sharpe and Skakkeback, 1993; Skakkeback et al., 2001). It is certainly possible that sub-sets of the population could have increased susceptibility to EDs in prenatal life particularly if the maternal detoxification systems were impaired.

Raised in utero levels of xenoestrogens have been suggested as dysregulators of testicular structure and function ('testicular dysgenesis syndrome'). Increases in the prevalence of cryptorchidism and hypospadias have been reported, although this may reflect differences in registration guidelines (Dolk et al., 2004). There seems, however, to be general agreement on higher incidences of testicular cancer. The tumours are primarily of germ cell origin and their rate of diagnosis has increased by 2–4% per annum since the 1960s in the USA, Great Britain and the Nordic and Baltic countries (Toppari et al., 1995). It is unclear whether this reflects life-style changes or ED dysregulation of hormonal levels in utero. Despite improved diagnosis, death due to cancer of the prostate has also increased over the past three decades and again there may be an environmental component in its causation (Safe, 1995). In addition, EDs can affect female development and function. Naturally occurring compounds such as genistein and daidzein, the isoflavones found in soybeans and soy products, act as weak oestrogens, as do other compounds of this type. They have been proposed as being protective against breast cancer and peri-menopausal symptoms and do have effects in vivo as soy isoflavones have been shown to increase follicular phase length and delay menstruation in pre-menopausal women (Safe, 1995). However, they may not necessarily be beneficial as both classes of compound inhibit SULTs 1A1 and 1E1 (Harris et al., 2004, 2005). Consequently, their ingestion and absorption could cause an increase in local oestrogen levels. Most breast cancers are, at least initially, oestrogen dependent and a surge in levels would be expected to promote growth. Breast cancer is one of the most frequent tumours in women world-wide; the incidence varies but is believed to be lower in South East Asia where soy consumption is greatest although other dietary factors, e.g. the consumption of catechins present in green tea, may play a role. It has been suggested that exposure to organochlorine compounds such as DDT and PCBs may be a factor in the increased incidence of the disease, but this is still controversial (Dewailly et al., 1994). There is a weak association between exposure to

the oestrogenic diethylstilboestrol (DES) in utero and the subsequent development of breast cancer (Colton and Greenberg, 1993), so it is not impossible that high levels of EDs before birth could be a factor in the disease aetiology. Epidemiological studies suggest that high levels of soy isoflavonoids may protect against breast cancer if they are part of the diet from infancy but that there may be no beneficial effect, or even an adverse effect, if they are introduced later in life (Cornwell et al., 2004). In both men and women, there appear to be ‘critical windows of exposure’, either pre- or peri-natal, which are periods of sensitivity to hormonal dysregulation by environmental contaminants.

10. Are EDs a risk to humans?

At present, the best ‘guestimate’ is probably “we know that EDs affect all phyla studied to date.” Phytoestrogen signalling for recruitment of soil bacteria for symbiotic nitrogen fixation was recently shown to be disrupted by ED pesticides. This is an ancient pathway (Fox et al., 2004). Compounds that can affect any species can potentially affect human beings. What we do not yet know is at what levels. The human race has active detoxifying enzyme systems, so is potentially at less risk from environmental toxins apart from those, such as PCBs, where the metabolites also give rise to adverse effects. Rodents, such as rats, are not particularly good animal models for reproductive toxicity in man (rabbits or pigs would be better). Nevertheless, if rats and humans have comparable pathways, the responses may be comparable qualitatively if not quantitatively. Probably the real dangers to man are not the obvious reproductive toxicology effects. Damage to neuronal development or the immune system could result in long-term dysfunction in later life. Both seals and polar bears have altered T-cell function in polluted coastal waters (Lie et al., 2004; Neale et al., 2002) while postnatal exposure to environmental levels of PCBs in Dutch school children was linked with reduced immune function (Weisglas-Kuperus et al., 2004). Neurodevelopmental effects of environmental contaminants have been reviewed recently (Rodier, 2004; Wormley et al., 2004); most studies suggest that learning and memory seem the parameters particularly likely to be affected. Animal models and testing are rarely valid here so that diffuse and subtle dysregulation of behaviour, memory or cognitive function might not be attributed as a response to ED exposure many decades previously. This is an area where there are no clear answers to difficult problems, partly because human developmental neurotoxicology is still not well understood. Another factor is that there is relatively little information on the body burden of EDs in human populations.

The intake of nonylphenols has been calculated to be ~7.5 µg/day in a German population with intakes for breast-fed and bottle-fed infants of 0.2 and 1.4 µg/day, respectively. Levels of EDs (a summation mainly of nonyl phenol, DEHP dibutylphthalate and diethylphthalate values) in Spanish whole milk samples were relatively high, ranging from 79.3 to 187.4 µg/kg so that the daily intake, particularly for children, may be significant (Casajuana and Lacorte, 2004). Analysis of urinary phthalate metabolites (thus avoiding any environmental contamination by the parent compounds) in a northern Bavaria (Germany) pop-

ulation showed wide ranges of concentrations. Nevertheless, the median concentration of the DEHP metabolite 5-hydroxy-MEHP was 46.8 µg/l with a range of 0.5–818 µg/l and the median concentration of mono-*n*-butylphthalate was 181 µg/l (Guenther et al., 2002). Measurements on cord blood from Italian neonates found DEHP or MEHP in 77% of samples, with mean concentrations of 1.19 ± 1.15 and 0.52 ± 0.61 µg/l, respectively (Koch et al., 2003). Analysis of maternal and neonatal plasma similarly confirmed that exposure to phthalates begins in utero and reflects the levels found in the mother (Latini et al., 2003a). Phthalate contamination is ubiquitous so these values are probably representative of Europe in general.

A further problem is that we do not know whether mixtures of different EDs (the so-called ‘cocktail effect’) are synergistic. EDs with the same mode of action are generally assumed to behave additively but there are few examples of this being tested (Latini et al., 2003b). We do not understand whether combinations of, for example, high levels of flavonoids from the diet or from dietary supplements, could increase responsiveness to ED action, particularly in susceptible sub-groups or in those exposed at critical stages of development. Levels of environmental organochlorine compounds in the diet have been estimated to be about 100 million times lower than levels of oestrogenic flavonoids but it is possible that the combination could be synergistic (Amaral Mendes, 2002). A series of research programmes funded by the European Commission under the ‘umbrella’ of the CREDO consortium (www.credocluster.info), aims to find answers to some of these questions. Researchers are studying brominated fire retardants and plasticisers, with a range of other EDs and investigating a series of topics including effects on reproduction, steroid metabolism, bone growth and toxicity to a variety of organisms. Long-term, the answers from these projects may further our understanding of the modes of action of EDs and hence a better analysis of their risks to man. Those investigators who are carrying out research in this field also aim to devise a range of in vitro tests which will enable us to diagnose ED potential in an industrial chemical or drug before it reaches the market (www.endomet.bham.ac.uk). Prevention will always be preferable to cure and technically much easier to achieve.

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