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Endocrine disruption: Fact or urban legend?

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HIGHLIGHTS

- We review the international definitions of endocrine disruptors (EDs).
- We discuss the association of EDs with the testicular dysgenesis syndrome.
- We discuss the pivotal role of potency in the safety assessment of EDs.
- We discuss additive effects of human simultaneous exposure to several EDs.
- We discuss whether human exposure to chemical EDs poses a human health risk.

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ABSTRACT

Endocrine disruptors (EDs) are substances that cause adverse health effects via endocrine-mediated mechanisms in an intact organism or its progeny or (sub) populations. Purported EDCs in personal care products include 4-MBC (UV filter) or parabens that showed oestrogenic activity in screening tests, although regulatory toxicity studies showed no adverse effects on reproductive endpoints. Hormonal potency is the key issue of the safety of EDCs. Oestrogen-based drugs, e.g. the contraceptive pill or the synthetic oestrogen DES, possess potencies up to 7 orders of magnitude higher than those of PCP ingredients; yet, in utero exposure to these drugs did not adversely affect fertility or sexual organ development of offspring unless exposed to extreme doses. Additive effects of EDs are unlikely due to the multitude of mechanisms how substances may produce a hormone-like activity; even after uptake of different substances with a similar mode of action, the possibility of additive effects is reduced by different absorption, metabolism and kinetics. This is supported by a number of studies on mixtures of chemical EDCs. Overall, despite of 20 years of research a human health risk from exposure to low concentrations of exogenous chemical substances with weak hormone-like activities remains an unproven and unlikely hypothesis.

Damnant quod non intellegunt.
They seek condemnation of what they do not understand. (Cicero)

It has been understood for a long time that a high consumption of hormonally active plant constituents, such as coumestrol or daidzein contained in clover, can adversely affect reproduction in domestic animals, up to induction of permanent infertility (Lindner, 1976). Therefore, it is not surprising that high doses of industrial chemicals, such as phthalates, chlorinated compounds as well as numerous other substances were also found to adversely affect reproduction in laboratory animals. From the early 1990s to the present, additional concerns have been raised that other man-made chemicals, such as phthalates, polychlorinated biphenyls or alkylphenols may also affect the reproduction in humans, wildlife or aquatic organism by disrupting their endocrine functions, e.g. hormones secreted by human ovaries, testes, thyroid or other organs (Nilsson, 2000; Safe, 1997, 2004). Thus a novel category of potentially hazardous substances, *endocrine disruptors* or *endocrine modulators* was born, although the exact definition of the meaning of these terms is still debated and somewhat unclear (Foster and

Abbreviations: BfR, Bundesamt für Risikobewertung (Berlin, Germany); DES, Diethylstilbestrol; DEP, diethylphthalate; ECETOC, European Centre for Ecotoxicology and Toxicology of Chemicals; ED, Endocrine Disruptor; EDC, Endocrine Disrupting Chemical; EFSA, European Food Safety Agency; EPA, US Environmental Protection Agency; EU, European Union; GLP, Good Laboratory Practice; IPCS, International Program on Chemical Safety; 4-MBC, 4-methylbenzylidene camphor; PCP, Personal Care Products/Cosmetics; TDS, Testicular Dysgenesis Syndrome; WHO, World Health Organisation.

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Agzarian, 2008). Indeed, the European Union, the WHO and the US EPA employ slightly different definitions thereby adding to the breadth of interpretational room for any data generated. Special concerns have been raised by some scientists and environmental activists that human exposure to hormonally-active ingredients used in personal care products/cosmetics with potential hormonal activity, such as parabens, phthalates or certain ultraviolet filters, may affect human endocrine systems and pose a risk to human health (Witorsch and Thomas, 2010). For example, it has been suggested that human exposure, in particular pre-natal exposure of the human foetus, to such substances may affect human semen quality, produce or contribute to male infertility, birth defects in male infants, breast and testicular cancers, obesity and other adverse health effects.

Given that chemicals with potential hormone-like activity, in particular personal care product ingredients, have a minute potency when compared with that of actual, mammalian hormones, such as oestradiol, novel hypotheses were developed that human exposure to mixtures of a multitude of weakly active substances may produce additive or even synergistic effects and yet pose a risk to human health (Witorsch, 2002a; Myers et al., 2009). Considering these hypotheses, we attempted to summarise the present state of knowledge on Endocrine disruptors and to review whether ingredients of personal care products/cosmetics may produce adverse human health effects secondary to endocrine disruption.

1. What are "Endocrine disruptors"?

The Endocrine system is the term for multiple and diverse hormonal systems in the mammalian organism, such as thyroid hormones, hormones originating from the pancreas, ovaries, testes, adrenals or the brain. There have been a number of definitions of what is an "Endocrine disruptor" (ED). The most commonly agreed are the following: *An ED is an exogenous substance that causes* ***adverse health effects*** *in the intact organism or its progeny, secondary/consequent to changes in endocrine function*"(Weybridge, 1996). A similar definition has been chosen by the World Health Organisation's International Program on Chemical Safety: *An endocrine disrupter is 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)populations* (WHO/IPCS, 2002). A joint expert group of the German Bundesamt fuer Risikobewertung (BfR) and UK health authorities (UK-BfR) regulatory expert group proposed an identical definition: *An ED is 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) populations* (BfR, 2011). The most recent definition issued by the EU is that *an ED is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function* (EU, 2012); this is also consistent with the recent definition by the European Food Safety Agency (EFSA, 2013). The US EPA defined EDC as follows: *Endocrine disrupting chemicals (EDCs) have been defined as exogenous agents that interfere with the production, release, transport, metabolism, binding, action, or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes* (EPA, 2012).

It is important to note that all major international definitions stipulate that an EDC must cause adverse effects via an endocrine-mediated mechanism in an intact organism. A common definition of what constitutes an adverse effect has been proposed by the BfR/UK expert group, the WHO IPCS, ECETOC and independent authors (BfR, 2011; WHO/IPCS, 2002; ECETOC, 2009; Lewis et al., 2002). All current definitions agree that the definition of an "adverse effect" means toxicity, i.e. pathology or functional impairment. Therefore, only a substance that produces *toxicity* in an intact organism via a hormonal or hormone-like mechanism represents a genuine ED.

Often substances have been reported to be ED based on the results of screening tests. Indeed, a considerable number of in vitro (sub-cellular or cellular) and in vivo (animal) screening tests for hormone-like activities of substances have been developed (Borgert et al., 2011). However, these tests were established for the purpose of screening, i.e. in order to prioritise toxicological testing of substances that may possess hormonal activities. Given that screening tests do not identify toxicity, they cannot determine whether a substance is an ED or not. Screening tests do not even assure that a substance will produce a hormonal activity in humans or other organisms; they merely suggest that the test substance may have such a potential. Therefore, when a substance produces changes in hormone-related parameters in screening tests, this means that the test substance has a biological activity, but it does not mean that it is toxic or is an ED. This view is also supported by a recent position of the European Food Safety Agency (EFSA, 2013). Indeed, there are more than a thousand natural or synthetic substances that have been found to be positive in screening assays and to possess weak hormone-like activities without causing actual toxicity at the individual or the population level. Weak hormonal activity can be advantageous, detrimental or neutral for the organism. To illustrate this: a change in room temperature, a meal or daylight may induce changes in circulating levels of hormones, such as thyroid hormones, insulin or melatonin, respectively (Foster and Agzarian, 2008). This does not mean that these innocuous factors should be considered to be EDs.

Similarly, our food is full of hormonally active substances: for example, soy contains substances (isoflavones) that possess powerful oestrogenic activity in screening assays, which have been shown to produce adverse reproductive effects in animal toxicity studies (Delclos et al., 2001; McClain et al., 2007). Thus, by definition, soy isoflavones, such as genistein, are genuine EDs. However, isoflavones do not produce oestrogenic effects in humans or non-human primates at dietary levels (Cline et al., 2001). Moreover, Asian populations with a high dietary intake of soy or soy-based food tend to have lower cancer rates of reproductive organs or breast, when compared with European or US populations (Peeters et al., 2003), but do not present an increased incidence of lower sperm count or TDS (testicular dysgenesis syndrome). It has been suggested that phytoestrogens may be useful for the prevention of breast and other cancers (Humphrey, 1998; Mense et al., 2008). Natural oestrogens or similar hormone-like substances are contained in clover, hops, Brussels sprouts, beer, wine, walnuts, linseed and many other plant foods (Kurzer and Xu, 1997). It has been known for centuries or longer that grazing sheep or cattle on clover may result in infertility, the so-called *clover disease*. Today, we know that this is due to coumestrol and daidzein, natural constituents of clover and natural contraceptives (Lindner, 1976; Oellermann et al., 1987). Given that clover or soy phyto-oestrogens have oestrogenic activity in vitro and been shown to adversely affect reproduction in animals, they should be considered genuine EDs – although, at normal concentrations present in animal feed or human food, there is no evidence that they pose a risk to health. A human health risk from soy isoflavones is also reduced by the fact that humans have a greater ability to than laboratory rodents to detoxify ingested isoflavones via glucuronidation in the gut wall or the liver (Pritchett et al., 2008; Setchell et al., 2008; Rozman et al., 2006). However, these examples demonstrate that mammalian organisms do not differentiate between natural and synthetic substances with respect to potential adverse effects. Overall, hormonal activity of a substance represents a biological activity or a mechanism

that can be good, bad or neutral for the intact organism. Hormonal activity on its own has little to do with hazard (toxicity) or even less suggests a health risk for the intact organism unless it leads adverse outcomes, for example carcinogenic, reproductive, or developmental effects that are routinely considered in reaching regulatory decisions (Dekant and Colnot, 2013; Testai et al., 2013; EPA, 2012).

2. Is there an association of endocrine disruptors and breast cancer?

Certain persistent organochlorine substances, such as pesticides (DDT) or polychlorinated biphenyls (PCB), have been shown to have a weak potential for hormonal activity. These substances were never used in Personal Care Products and have been banned in the US, Europe or Japan for more than 30 years, although declining residues continue to be present in the environment. It has been suggested that residues of these substances in the human organism combined with their hormonal activity may play a role in the aetiology of breast cancer. Indeed, in 1992, US researchers reported elevated levels of PCBs and DDT in a relatively small group of women with breast cancer; subsequent US studies suggested a slight association between breast cancer and elevated serum DDT, but not serum PCB, levels (Krieger et al., 1994; ACSH, 1999). However, follow-up studies on a large number of US breast cancer patients found no correlation between the human body burden of organochlorine substances and breast cancer. Subsequent official US reviews of the association of breast cancer, endometrial cancer and endometriosis with organochlorine residues concluded that no such relationship could be supported by the existing evidence (Ahlborg et al., 1995; Safe, 1997).

In 2004, a study reporting paraben residues in human breast tumour tissues re-opened the media attention and subsequent debate about a possible link between endocrine disruption and breast cancer. Given that some long-chain parabens possess a very weak potential for hormonal activity, the authors of the study suggested that parabens contained in underarm deodorants may migrate into the breast and therefore may have a causal role in the aetiology of breast cancer (Darbre et al., 2004). However, that study had serious weaknesses: the detected paraben residues were minute (in the range of nanograms per gram of tissue), the study did not investigate paraben levels in healthy tissues and there is no known direct transport mechanism of externally applied underarm substances into the breast. Finally, the study detected the same parabens in tumours and blank solvents that were used for tissue extraction. Moreover, the authors failed to notice that deodorants do not contain parabens since they require no preservatives due to their high content of aluminium. The study and its claims were rejected as flawed by a number of experts and international health authorities who concluded that there is no scientific evidence linking underarm deodorants or parabens with breast cancer (Witorsch and Thomas, 2010). This view is supported by the results of a large epidemiological investigation that showed no evidence for an increased risk of breast cancer and underarm antiperspirant use (Mirick et al., 2002). Nevertheless, an urban legend was born.

Indeed, long-chain parabens (butylparaben, propylparaben) possess extremely weak estrogenic potencies, i.e. about 10,000- to 1,000,000 lower than that of oestradiol or synthetic hormones, such as ethinylestradiol, an active agent of contraceptive drugs (Routledge et al., 1998; Golden et al., 2005; CIR, 2008). Yet, the human contraceptive pill is used daily, chronically, and contains large doses of highly potent oestrogens and other hormones. However, use of the contraceptive pill has not been associated with a significant increase in the risk of breast cancer. It is therefore scientifically implausible how the minute activity of parabens could pose a risk to human health, particularly when they are contained in low concentrations in products that are applied to human skin. In this context it should also be mentioned that the incidence of breast cancer in the US and other industrialised countries has been stable since the 1980s, which is also inconsistent with the alleged human exposure to EDCs (SEER, 2010; Ahlborg et al., 1995; Safe, 1997).

3. The hypothesis of the "Testicular dysgenesis syndrome" (TDS)

A current hypothesis has suggested that, in modern industrial societies, human male fertility is declining, whereas the incidence of diseases or birth defects of the male reproductive system is increasing. The hypothesis that human sperm count is declining whereas the incidence of testicular cancer, cryptorchidism (undescended testis) and hypospadias (abnormally placed urethral opening at the underside of the penis) is increasing and that these changes are associated with human exposure to hormonally active chemicals. The hypothesis was popularised in 1992, when a group of Danish researchers reported a 50% decrease in sperm count for the period of 1938–1992 (Carlsen et al., 1992), although that study had significant weaknesses (Handelsman, 2001; Fish, 2000 and 2008). In the meantime, this hypothetical syndrome has been named the "Testicular dysgenesis syndrome" (TDS). It postulates that, in Western industrialised countries a) human male fertility, in particular sperm count, has declined and continues to decline, b) the incidence of human testicular cancer is increasing, c) the incidence of cryptorchidism and d) hypospadias in newborn male infants has increased (Thorup et al., 2010). An additional hypothesis is that the increased incidence in the TDS may be due to chemical EDC in the human environment.

First, it should be realised that sperm count is not equal to male fertility. It is a common fallacy that male fertility can be measured by counting sperm. Sperm count/output is actually a surrogate variable for male fertility, but not necessarily a good one (Handelsman, 2001). For example, studies in Sweden (Akre et al., 1999; Scheike et al., 2008) or the UK (Joffe, 2000) suggested that human fertility has increased in the past decades. Second, there is no evidence for a general decline in sperm count (Saidi et al., 1999; Fish, 2000, 2008; Thorup et al., 2010; Itoh et al., 2001; Handelsman, 2001; Safe, 2004, 2005; Greim, 2005). Although some studies found a decline (Carlsen et al., 1992; Rolland et al., 2012), others found an increase in sperm count (Saidi et al., 1999; Fish, 2008). A recent study, one of the largest, longest and best-controlled investigations ever performed on this issue, found no decrease, but a slight, but significant increase in the mean sperm count of nearly 5000 Danish military recruits over the period of 1996–2010 (some study data first reported by Bonde et al., 2011). Although the results of that study have been available for several years, the complete study was only published in 2012 (Jorgensen et al., 2012), possibly in response to pressure from the scientific community (Anon., 2011; Wilcox, 2011; Bonde et al., 2011). Nevertheless, a number of far smaller studies that claimed declining sperm counts were published by the same researchers – and widely reported by the media.

Clearly, such a "publication bias" distorts the public risk perception and may result in conjuring imaginary health risks and disease associations with exposure to chemicals. Given that current data suggest that sperm count in Denmark had remained unchanged or even slightly improved over the past 15 years, this finding is now consistent with similar data from Japan, Sweden and the US, which showed that sperm count has remained stable for the past 20 years (Itoh et al., 2001; Axelsson et al., 2011; Fish, 2008; Saidi et al., 1999). Here it also should be considered that sperm counts reported prior to the 1990s must be regarded with great caution due

to methodological shortcomings and non-standardised methods (Handelsman, 2001; Fish, 2008). Even in industrialised countries a lack of compliance with standardised sperm morphology/count methods was reported as recently as 2012 or 2005 (Mallidis et al., 2012; Riddell et al., 2005). Finally, the notion that prenatal oestrogen exposure has adverse effects on male fertility has been refuted by studies on boys born to women exposed to high oral DES doses during pregnancy. Neither fertility nor sperm output were adversely affected despite massive in utero oestrogen exposure, although minor urogenital malformations did occur in this population (Leary et al., 1984; Handelsman, 2001). It is noteworthy that there was an approximate 14-fold difference between the highest and the lowest clinical dose of DES; reproductive malformations were observed only among the offspring of women who received high-dose regimens (Borgert et al., 2012).

Moreover, there is no scientific evidence for a general increase in the incidence of cryptorchidism or hypospadias in male infants; in addition, it has been argued that these two pathologies are caused by different mechanisms, which cast even more doubt on a common origin or a common causal agent (Thorup et al., 2010). An increased risk of hpospadias or cryptorchidism appears to be associated with increasing maternal age and body mass (Fish et al., 2001; McGlynn et al., 2006), whereas a high incidence in hypospadias has also been linked with a maternal low protein or vegetarian diets during pregnancy (Akre et al., 2008; North and Golding, 2000). On the other hand, there appears to be an increase in the incidence in testicular cancer in some Western countries, although the incidence in other industrialised countries and regions, such as Asia, appears to be stable. Although maternal body weight may play a role in the aetiology of testicular cancer (Aschim et al., 2005) the reason for this discrepancy remains unknown; however, there is no evidence for a causal relation to chemical EDCs, to the contrary: for example, a large follow-up study in more than 1,300,000 Danish man found no correlation between testicular cancer and peri-natal oestrogen exposure (Ramlau-Hansen et al., 2009). Similarly, a review of 81 epidemiology publications concluded that *there is no strong epidemiological evidence to indicate that prenatal exposure to oestrogen is linked to disturbed development of the male reproductive organs* (Storgaard et al., 2006). This notion is consistent with observations in a population of males that were exposed in utero to maternal doses of DES, which observed no major increase in the incidence of testicular cancer (Leary et al., 1984). Overall, it is uncertain whether the TDS actually exists and even more uncertain that synthetic chemical EDCs are associated with it (Thorup et al., 2010; Safe, 2005).

4. Personal care product ingredients: purported endocrine disruptors?

A series of substances in personal care products have been branded as putative EDC. They include solvents, such as diethyphthalate, preservatives (long-chain parabens, triclosan), fragrances (polycyclic musks), UV filters (4-methylbenzylidene camphor) or long-chain phthalate esters (Witorsch and Thomas, 2010). It is true that certain long side-chain phthalates, such as diethylhexyl- or butyl phthalates, have weak hormonal activities and may affect male fertility in rats – when given at high oral doses. In contrast, diethylphthalate, which has been widely used as a vehicle in fragrances, is devoid of hormonal activity or other significant toxicities. Diethylphthalate was shown to be non-toxic in a large number of safety evaluations and was rated to be safe for use in cosmetics by international expert groups, such as the EU SCCS or the US Cosmetic Ingredient Review (Witorsch and Thomas, 2010). However, the chemical name *phthalate* made this substance target of attacks by NGOs due to an alleged endocrine disrupting activity, in other words: health risk assessment based on the name *phthalate*.

In 2001, the UV filter 4-methylbenzylidene camphor (4-MBC) was reported to be active in vitro as well as in vivo (uterotrophic test in immature female rats) screening studies for oestrogenic activity (Schlumpf et al., 2001). However, when the substance was tested under conditions of Good Laboratory Practice (GLP) in a complete 1-generation reproductive toxicity study, no adverse effects were found (Broschard et al., 2004). Therefore, per definition, 4-MBC is not an ED. In addition, an in-depth evaluation of the metabolism of 4-MBC in humans and rats after dermal application as well as a toxicokinetic-based safety assessment concluded that the substance poses no health risk for humans (Schauer et al., 2006).

Long side-chain parabens, such as butylparaben, but not methyl- or ethylparaben, have also been reported to possess weak oestrogenic activity in rats when given subcutaneously (but not when given orally or dermally) at high doses (Routledge et al., 1998). Other studies reported that repeated oral doses of butyl- and propylparaben adversely affected male fertility parameters in juvenile rats (Oishi, 2001). However, when the latter studies were repeated in larger investigations and under conditions of Good Laboratory Practice, no effect was found at oral doses of up to 1000 mg/kg/day suggesting that propyl- or butyl parabens have no ED activity (Gazin et al., 2012; Hoberman et al., 2008).

In addition, it has been shown that whole-body application for two weeks of a cream containing 2.0% butylparaben (10 times the maximum concentration permitted in the EU and applied at 2 mg/cm^2) did not affect reproductive and thyroid hormone levels in humans (Janjua et al., 2007). A study in 332 consumers (post-menopausal women) found no presence of butyl- or benzyl parabens in their plasma (Sandanger et al., 2011). Subsequently, the US Cosmetic Ingredient Review reviewed the safety of parabens in-depth and concluded that all parabens are safe as used in personal care products (CIR, 2008). In contrast, in the EU, the maximal content of propyl- and butylparabens in PCP were limited to concentrations of 0.2%. In addition, there is growing evidence that parabens, when used in products applied to the skin, are hydrolysed (de-toxified) in human and animal skin resulting in para-hydroxybenzoic acid (Jewell et al., 2007; Aubert et al., 2012; CIR, 2008), a natural substance that is ubiquitous in plants, vegetables and human food. Para-hydroxybenzoic acid also occurs in human breast milk at concentrations of approximately 600 μg/kg, whereas infant formulas contain up to 3590 μg/kg (Li et al., 2009).

Taking into account that it is unlikely that the human organism is systemically exposed to parabens after application of paraben-containing PCP products to the skin, the potential human health risk from parabens in PCPs should be rated negligible or absent. In this context, it is interesting to note that methylparaben is a natural, sex-attractant pheromone in female dogs (Goodwin et al., 1979). Thus it may be argued that, at least in canines, certain parabens further reproduction rather than impede it.

5. Hormonal potency: a key topic of safety assessment

The key question on the safety of substances with a hormone-like activity is their individual hormonal potency. The importance of potency of potential EDC for their human and environmental safety assessment has been reviewed by several authors and expert groups (Calabrese et al., 1997; Borgert et al., 2013; Fegert, 2013; Testai et al., 2013; Dietrich et al., 2013). To illustrate potency aspects: both cyanide and table salt can be toxic, but cyanide is far more potent than table salt. Consequently, using common sense or *risk management*, people tend to be more cautious when they handle cyanide than when they use table salt. In analogy, oestradiol, the mammalian female sex hormone or synthetic oestrogens contained in oral contraceptives are extremely potent and active in microgram doses or when present in human blood at ng/mL levels, i.e. in

Table 1
Comparative oestrogenic potency of natural or synthetic substances in the rodent uterotrophic assay after oral doses (adapted from Golden et al., 2005; Nilsson, 2000; Witorsch and Thomas, 2010).

Substance	Use/Origin	Effective dose (mg/kg/day)	Relative potency
Diethylstilbestrol (DES)	Drug	0.0001	3,000,000
Ethinylestradiol	Contraceptive pill	0.0003	1,000,000
Estrone	Human oestrogen	0.0012	250,000
Coumestrol	Legumes (clover)	0.03	10,000
Genistein	Soybeans	8	37
Daidzein	Soybeans	12	25
4-MBC	UV filter	300	1.0
Butylparaben	Preservative	600[a]	0.5
Benzylparaben	Preservative	2500	0.12

[a] Subcutaneous doses, rats.

vivo. Indeed, natural or synthetic hormones (e.g. ethinylestradiol) are 10,000 to 1,000,000-fold more potent than man-made chemicals with an oestrogenic activity, such as long-chain parabens or ultraviolet filters (Golden et al., 2005; Witorsch, 2002a; Witorsch and Thomas, 2010; Table 1). These differences in potency are by several orders of magnitude higher than those between the toxicity of table salt and cyanide. When imagining that one oestrogenic potency unit corresponds to one horse power, ethinyl oestradiol (ingredient of the contraceptive pill) would have the power of a super tanker, coumestrol (clover) that of a bomber, genistein (soy beans) that of a small car, whereas butyl- or benzylparaben would be in the power range of that of a kitchen mixer or a children's bicycle, respectively (Fig. 1).

Here it should be noted that butylparaben, although falsely branded to be an "Endocrine Disrupter", only showed extremely weak potential for oestrogenic activity (rat uterotrophic test) when administered subcutaneously (injected under the skin) at doses of 800 mg/kg and higher (Routledge et al., 1998). This would correspond to a human *subcutaneous* dose of 48 grams in a 60 kg human being in order to produce a potential activity (corresponding to 50 kg of a cream, containing 0.2% of butylparaben), not to mention that humans are less sensitive to some hormonal effects than rats (Borgert et al., 2012; Witorsch, 2002b)). When butylparaben was given orally to rats or applied to the skin of rats it had no oestrogenic

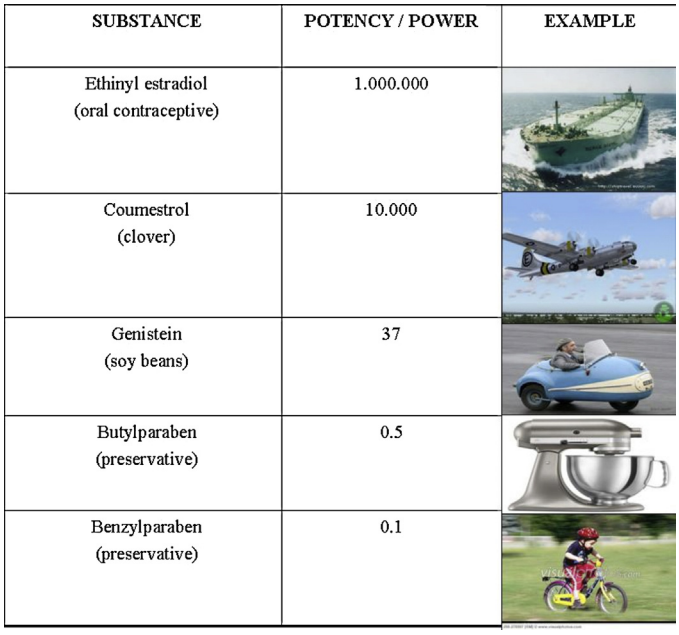

Fig. 1. Assuming that one oestrogen potency unit (see Table 1) corresponds to one horse power, the power/potency of some of the substances in Table 1 may be ranked as follows.

activity (Routledge et al., 1998). In a reproductive study in young male rats, butylparaben produced no adverse changes in reproductive organs or reproductive hormone levels at doses exceeding 1000 mg/kg/day (Hoberman et al., 2008). Considering this, it is biologically implausible how butylparaben, when included at 0.2% in a cream, could have any effect at all or even pose a human health risk. Overall, when taking into account their limited skin penetration, their metabolism in the skin and their minute oestrogenic potency of substances used as cosmetic ingredients, a risk to human health may be excluded altogether (Golden et al., 2005; Witorsch and Thomas, 2010). This view was also supported by the results of biomonitoring studies on actual blood levels of parabens in human consumers. Given that detected levels were absent or negligible, a hormonal effect, not to mention a human reproductive risk, may be clearly excluded (Sandanger et al., 2011). It has been argued that the potency of the most active environmental oestrogens would need to be at least 1000-times higher in order to present human reproductive risks (Borgert et al., 2012).

Oestrogen-containing drugs (e.g. the contraceptive pill) or the synthetic oestrogen DES possess potencies that are by 6 or 7 orders of magnitude higher than that of long-chain parabens or UV filters with "estrogenic activity" (Table 1; Fig. 1). Yet, prenatal in utero exposure of men to oestrogen drugs or DES did not adversely affect their fertility (Hemminki et al., 1998; Wilcox et al., 1995; Leary et al., 1984) or sperm parameters (Schumacher et al., 1981). It is also noteworthy that DES, although it is several times more potent than oestradiol, appears to have a no-effect level: human *in utero* exposure to maternal doses of a total of 1.4 g DES over 101 days (approximately 0.25 mg/kg/day), a huge dose in terms of oestrogenic potency, did not produce urogenital abnormalities or abnormal sperm parameters in male offspring (Fish, 2000). A no-effect level was also observed for ethinyl oestradiol (an active ingredient of the contraceptive pill): when injected in adult men at high doses (60 μg/day) it affected sperm motility and density; in contrast, 20 μg/day, still a huge dose in terms of hormonal potency, had no effect on sperm motility and density (Lübbert et al., 1992). Taking these data into account, the hypothesis that the negligible exposure of humans to chemicals of negligible hormonal potency could have an effect on human fertility is absurd defying a scientific basis as well as common sense.

6. Health risks of simultaneous exposure to several substances with weak hormone-like activities ("Cocktail Effects")

It has been hypothesised that, although individual man-made substances generally have a very weak hormonal potency, the combination of several weakly acting substances may be additive or even synergistic and thereby yet produce adverse effects on the organism. This hypothesis has been raised as early as 1996 when Arnold et al. (1996) claimed that mixtures of several chemicals had

synergistic potency on human oestrogen receptors. These data produced a major debate by the scientific community and the media (Anon., 1997). However, the results could not be reproduced in other laboratories because the data were fabricated, and the previous article was withdrawn by the authors (McLachlan, 1997). Recently, the hypothesis of a possible addition or synergy of low doses of EDs has been raised again (Vandenberg et al., 2012; Zoeller et al., 2012). These, albeit hypothetical, effects were named *Low-Dose Effects*, *Dose Addition* of substances that produce *Common Adverse Outcomes* (*DA-CAOS*) or *Cocktail Effects*.

However, these effects are highly improbable, if not impossible, on the basis of theoretical as well as practical considerations (Borgert et al., 2005, 2012; Rhomberg and Goodman, 2012). First of all, there is clear evidence that reproductive toxins including those with a hormone-mediated mechanism have a threshold of adversity (Piersma et al., 2011). To be additive, they would have to possess exactly the same mode of action (Borgert et al., 2005). Moreover, there is a multitude of direct or indirect mechanisms by which substances may affect hormones or produce a hormone-like activity. For example, substances that produce an oestrogen-like effect may have an affinity for cellular oestrogen receptors or oestrogen sub-receptors; they may have agonistic or antagonistic properties, they may have oestrogenic, androgenic or anti-androgenic activity or they may produce indirect effects, such as direct toxicity to, or stimulation/inhibition of oestrogen-generating tissues. Even when the same target organ or hormonal system is affected, the mode of action of adversity may be quite different: for example, in rats, there are a multitude of mechanisms whereby substances may affect the thyroid or thyroid hormone levels, although many of these mechanisms are not relevant for man. In addition, it has been recognised that the rat is an inadequate model to predict adverse effects on thyroid hormones of drugs in man (Wu and Farelly, 2006).

In the organism there are many different hormones that may act on different receptors or sub-receptors. To illustrate this, not even the natural oestrogen oestradiol and the powerful synthetic oestrogen DES share an identical mode of action (Safe, 1998). Even after simultaneous uptake of different substances that have the same or a similar mode of action, the possibility of additive or cocktail effects is reduced by different absorption, metabolism and kinetic pathways, which are never identical for different substances. Moreover, there are major quantitative and qualitative differences in the affinity or activity between weak and strong ligands (substances with affinity to cell receptors) to or on cell receptors. Based on pharmacological principles, weak ligands will only occupy and trigger cell receptors when present at high, near-toxic concentrations, but they have no effect when present at small concentrations. Overall, the presence of myriads of weak hormone receptor agonists or antagonists in the environment and food is not expected to achieve physiological significance, since many would be competitive agonists/antagonists their low potencies would preclude activity via the receptor (Borgert et al., 2005, 2012).

The complexity of these interactions has been demonstrated in a number of studies. For example, at high concentrations (EC_{25} to EC_{50}) a mixture of different-, anti-androgenic phthalates and bisphenol A had additive androgenic effects on MDA-kb2 cells, whereas low concentration mixtures ($<EC_{25}$) had antagonistic activity (Christen et al., 2012). Despite the fact that different mixture ratios near the observable response range can be interpreted as concentration additive, there is no evidence of additivity at doses in the range humans might be exposed, and no theoretical justification for extrapolating such data to human exposure levels (Borgert et al., 2012). A similar phenomenon was confirmed in another study on mixtures of weakly estrogenic UV filters in fish: mixtures containing high concentrations (EC_{10} to EC_{30}) of individual UV filters showed additive activity, whereas mixtures containing low concentrations (range: NOEC to EC_{05}) had a lower activity than predicted by the dose addition model, which suggests antagonism (Kunz and Fent, 2009). No additive effects were observed in rats after oral administration of combinations of high doses of ethinylestradiol (a powerful oestrogen contained in contraceptive pills), and genistein, an oestrogenic isoflavone contained in soy (Takagi et al., 2004). An additive effect was observed when high doses of genistein were combined with therapeutic doses of ethinylestradiol, whereas low doses showed no additivity (Charles et al., 2007). This observation suggests that additivity may be constrained to substances with moderate to high potency when given near their individual response level, and would be unlikely to occur with substances possessing low potency or when given at low doses (Borgert et al., 2012). Since human exposures are typically orders of magnitude below the observable response range, most mixture studies are not relevant to human health risks.

No evidence of synergy was found when weakly estrogenic hydroxylated polychlorinated biphenyls and pesticides were tested in combination (Arcaro et al., 1998). Another large study investigated binary mixtures of six “endocrine disrupting” chemicals (organochlorine compounds, phytoestrogens and actual hormones) that were administered at five different concentrations to bobwhite quail; some combinations were additive, others were antagonistic. Although group sizes were low and therefore, conclusions uncertain, no indications for synergy were detected (McMurry and Dickerson, 2001).

Another study investigated oestrogenic responses to mixtures of synthetic chemicals combined with phytoestrogens at various dose levels. As would be predicted by theoretical pharmacological considerations, low concentrations of the synthetic chemical mixtures failed to increase in vitro or in vivo estrogenic responses relative to phytoestrogens alone. Significantly increased responses occurred only when each synthetic chemical in the mixture was near or above its individual response threshold. In vitro, high doses of chemicals and phytoestrogens produced greater than additive responses, whereas mixtures of synthetic chemicals produced less than additive responses in the absence of phytoestrogens. In vivo, the combined effects were consistent with additivity. The study concluded that mixture effects are likely to be of concern only when mixture components are present at or near their individual response thresholds, and that extrapolation of mixture effects from in vitro to in vivo should be approached with caution (Charles et al., 2007).

Similarly, in another study, rat uterotrophic tests on a number of mixtures of synthetic chemicals (nonylphenol, octylphenol, β-hexachlorocyclohexane, methoxychlor, bisphenol A, dibutylphthalate) in combination with estradiol showed no additive or synergistic effects, whereas some combinations of phytoestrogens (coumestrol, genistein, narigenin, catechin, epicatechin, quercetin) with estradiol acted additively. Interestingly, all synthetic chemicals, when tested alone were inactive in the rat uterotrophic test even at high dose levels relative to human exposure (Van Meeuven et al., 2007).

The results of 90 different studies on endocrine effects of chemical mixtures were recently reviewed by an expert group who concluded that synergy is extremely rare and that the amount of synergy at low doses, when occurring, did not exceed the levels predicted by additive models by more than a factor of four (Boobis et al., 2011).

Overall, there is no genuine in vivo evidence for additive or cocktail effects of small doses of man-made, chemical substances with hormonal activity. Taking into account the fact that animals and humans are exposed to thousands of natural hormone-like substances in their food and environment that are capable of overt hormone-related toxicity at high levels of exposure in vitro, it is difficult to explain how these species have survived if such substances

significantly add to or subtract from endogenous hormonal activity, which is vital for life (Borgert et al., 2012).

7. Discussion: facts versus fears

Labels like "Endocrine disruptor" or "hormone-like substances" are stigmatic terms; they sound dangerous, raise media attention and provoke human fears. Yet, in the absence of relevant human exposure and potency data, these terms are meaningless in terms of human health risks. Overall, the entire discussion whether man-made chemicals with hormone-like activity may pose a risk to human health has a paradoxical aspect: if such activities, however small, could actually pose a potential health risk, then it would make sense to worry about *all* substances that possess such activities, particularly when potent oestrogens, such as the contraceptive pill, are taken orally or when they are present in human food, such as phytoestrogens. To the contrary, a number of epidemiology studies suggest that the potent contraceptive pill or naturally occurring soy isoflavones or other phytoestrogens, pose no or negligible risk to human health or that of human progeny. Thus, it is difficult to conceive how synthetic substances that are not eaten and possess only a tiny fraction of the activity of pharmaceutical or some natural substances could be dangerous (see Fig. 1). Here it should be considered that average humans consume about 100 μg of oestrogen equivalents a day from natural sources (e.g. soy flavonoids), whereas chemicals, such as butylphthalate, in human food amount to about 0.02 μg oestrogen equivalents (Nilsson, 2000). Yet, activists and opportunistic and media-cited scientists focus on that tiny number. To put these figures into perspective: a single contraceptive pill contains the staggering amount of about 17,000 μg of oestrogen equivalents, reflecting the striking potency of genuine hormones.

Science is about establishing cause and effect, it is not about guessing. Scientists develop a hypothesis – substance x causes observation y - and then should rigorously test the hypothesis to determine whether it is valid or not. If the hypothesis is tested rigorously and cannot be refuted, it must be tentatively accepted that the hypothesis may be right (Taubes, 2012). On the other hand, if repeated testing fails to generate unequivocal support, the hypothesis should be viewed with scepticism. Let us put the man-made environmental disruptor hypothesis to the test: the hypothesis has now been evaluated experimentally and epidemiologically for nearly 20 years and no convincing evidence has been found of an actual decline in human fertility, and even less of a causal relation with synthetic hormonally active substances.

This raises another important issue: epidemiology attempts to determine the cause(s) of an established disease (Susser, 1991). Bacteria, viruses or exposure to toxic substances may cause human diseases. To illustrate this, in the 1950s, a causal relation was established for lung cancer and tobacco smoking. Indeed, lung cancer is a genuine disease with measurable frequency. Its incidence dramatically increased in the 50s, whereas cigarette smoking became increasingly popular in the preceding decades. Exposure was certain, given that tobacco smoke is directly inhaled into the lungs. Thus the hypothesis for a causal relation made biological sense and causality was confirmed by a number of subsequent investigations that involved millions of subjects unequivocally exposed to direct inhalation of tobacco smoke. But how can one determine a cause of a disease when the existence of the disease itself is uncertain? For example, the Testicular dysgenesis syndrome (TDS) is merely a hypothetical disease, in other words: nobody knows whether this disease exists or not - some experts in the field doubt whether TDS exists at all (Thorup et al., 2010). Scientifically and philosophically, the search for a *hypothetical cause* of a *hypothetical disease* makes no sense – would it not make more sense to first make sure that the disease actually exists, before spending millions on the quest of its cause? With good reason, the quest for environmental, man-made ED has rightly been titled by the European Molecular Biology Organisation as *A Cause without a Disease* (Breithaupt, 2004). Nevertheless, we are now witnessing the advent of a massive regulatory programme in search of a justifiable public health purpose (Gori, 2007). Finally, even when a substance is active in an in vitro or in vivo ED assay, it is generally very difficult to prove that the effect was actually caused by an endocrine mechanism, since a concomitant effect on endocrine function on its own is not proof of causality per se. As an example, many substances may affect the rat thyroid and rat thyroid hormones by a number of diverse modes of action many of which are not endocrine-relevant (Wu and Farelly, 2006). This poses an additional, yet unresolved, problem of how to prove that a substance is an ED in actual practice.

The hypothesis of and subsequent search for man-made (synthetic), chemical EDs in the environment, food or personal care products began in the early 1990s. Up to date, this research has spent hundreds of millions of Euros or Dollars of tax payer's money. In the EU alone, more than 150 million Euros have been spent on research into potential health risks of EDCs (Jensen and van Vliet, 2012). Given this large amount of research funding there may also be a vested interest of scientists in the ED field to keep the ED hypothesis on the agenda in order to stay in business.

In January 2013, a search in TOXLINE for the term "endocrine disruptors" yielded 4278 different articles (TOXLINE, 2013) reflecting the enormous amount of scientific research devoted to this topic. A search in MEDLINE for the term "endocrine disruption" listed 33 annual publications in 1992 with an increase to 290 in 2011 (MEDLINE, 2013). Taking into account the large resources spent on this topic, one should expect that, in the meantime, some EDs that cause actual human injury or disease should have been identified. However, this is not the case. To date, with the exception of natural or synthetic hormones, not a single, man-made chemical ED has been identified that poses an identifiable, measurable risk to human health (the adverse effects of iatrogenic DES were long known before the term *endocrine disruptor* was coined). Certainly, there has been much media hype about imaginary health risks from bisphenol A, parabens or phthalates. However, no actual evidence of adverse human health effects from these substances has ever been established. To the contrary, there is increasing evidence that their health risks are absent or negligible – or imaginary. It is interesting to note that even substances, such as long-chain phthalates, which were confirmed to be EDs (anti-androgenic) in the rat, were largely inactive in the mouse or in vivo *ex situ* human foetal testis (Johnson et al., 2012). Given that the human foetus appears to be at least an order of magnitude less sensitive to DES-induced reproductive tract malformations than the rat foetus (Borgert et al., 2012), such species differences raise doubt as to the relevance of current rat in vivo test data for humans who, in addition, are exposed environmentally to far lower levels of such substances than laboratory rats.

The largest human experiment on endocrine disruption included more than a billion women and has continued for almost half a century: the contraceptive pill. However, the contraceptive pill does no significant harm to human health, although it has a powerful oestrogenic activity, is taken daily and is used over long periods of time. It has been estimated that about 500 Million women in the EU and the US annually use oral contraceptives with approximately 5% or 25 million women per year becoming pregnant and unknowingly expose their foetuses to potent oestrogenic and progestin drugs (Takeda, 2001). Yet, no adverse effects were observed in men or women after previous in utero exposure to oestrogen-containing drugs (Hemminki et al., 1998; Storgaard et al., 2006). The contraceptive pill is a genuine Endocrine Disruptor: it affects human reproductive physiology and contains

oestradiols (natural or synthetic) that are toxic to reproduction when given at high doses. Human urinary excretion of residues of the contraceptive pill as well as natural human and animal oestrogens in water may also affect the environment and are thought to be responsible for the feminisation of male fish that has been observed in large rivers downstream the effluent water of major cities or mass production of farm animals (Vajda et al., 2011; Jobling et al., 2006). Interestingly, the contraceptive pill has rarely, if ever, been a target of the EDC-lobby. It is outside the scope of this paper to speculate about the reasons. Possibly, focusing on chemicals, and not pharmaceuticals provides the emotional argument that these are everywhere and everybody is exposed or attacking the comparatively tiny oestrogenic activity of some chemicals that produce no or negligible human exposure may be more politically acceptable than blaming the pill. Chemical substances are, after all, *perceived* to pose a human and environmental health risk, whether this is supported by scientific facts or not. Blaming the contraceptive pill for adverse environmental effects is certainly less opportune.

It is not surprising that, when it was realised that it is implausible that hormonally active chemicals pose a human health risk given the small human exposure and their negligible potencies, new hypotheses had to be invented in support of the notion that they pose a risk anyway, such as the *Dose Addition* of substances that produce *Common Adverse Outcomes* (*DA-CAOS*) (Kortenkamp and Faust, 2010) or *Cocktail Effects* (Vandenberg et al., 2012). Despite of heroic efforts to find in vivo observational validation for these hypotheses, the in vivo evidence continues to accumulate that these effects are absent at low doses/concentrations, which is consistent with pharmacological theory (Borgert et al., 2012; Rhomberg and Goodman, 2012). Another interesting hypothesis is the *non-monotonic dose-relationship*, which postulates that, for EDs, high-dose effects may be non-predictive for effects observed for low doses (Vandenberg et al., 2012; Myers et al., 2009). Although this hypothesis is consistent with the ideas of homoeopathy, it contradicts centuries of toxicological and pharmacological experience demonstrating that active substances produce a specific dose-response in the affected organism. There are some exceptions to this rule, although they are generally due to different mechanisms of the respective substance, such as hypervitaminosis A versus vitamin A deficiency, both of which can be teratogenic. Although non-monotonic dose response curves for hormonal effects had been postulated in a limited number of studies, the data could not be reproduced in larger in vivo studies that included adequate quality assurance (Dekant and Colnot, 2013; Dietrich et al., 2013). Here an interesting point may be raised: if non-monotonic dose-relationships were relevant to the safety assessment of man-made, chemical EDs, surely the same concept should also apply to the contraceptive pill or phytoestrogens: accordingly, could a single Chinese meal or a cup of coffee wreak havoc with our endocrine systems? Does this assumption appear realistic?

In our view, the EDC saga may be a political, rather than a scientific problem. Paradoxically, today soy oestrogens (isoflavones) are marketed in health food stores with the claim that they relieve menopausal symptoms. Yet, women in menopause have an increased risk of breast cancer, whereas isoflavones have a potential to promote mammary and endometrial carcinogenesis (Kakehashi et al., 2012). Soy-phytoestrogens have also been reported to inhibit the activity of anti-cancer drugs, which may be of particular concern for women with oestrogen-dependent breast cancer (Gallo et al., 2007). In rats, in utero exposure to high maternal doses of the soy phytoestrogens genistein has been shown to adversely affect reproductive parameters in their male and female offspring; thus soy isoflavones are genuine EDs (McClain et al., 2007; Delclos et al., 2001). At dietary levels, however, phytoestrogens do not appear to induce oestrogenic effects on the uterus of humans or non-human primates (Cline et al., 2001). Another interesting example of an endocrine-active substance is caffeine: caffeine was embryo- and foeto-toxic in rat reproduction and developmental toxicity studies, it affected sperm quality in mice, increased the incidence of tumours in endocrine organs (pituitary adenomas and mammary tumours in mice and rats), and was positive in an in vitro steroidogenesis assay. In addition, the margin of exposure for human coffee consumers (mean daily intake: 240 mg caffeine) relative to adverse effects observed in animals is as low as 15- to 35-fold (Bars et al., 2012). Very recently, another series of substances (vitamins C, B9, B6, B3, sucrose, caffeine, gingerol, xanthane gum, paracetamol and ibuprofen) were tested in a series of in vitro assays for endocrine activity. Paracetamol, gingerol, caffeine and vitamin C affected stereoidogenesis in vitro from 250, 25, 500 and 750 μM, respectively. Caffeine, when tested in vivo (rat pubertal assay) at dose levels relevant for human consumption affected vaginal opening, oestrus cycle and ovarian weight in females as well as plasma progesterone levels, prostate and seminal weights in males (Tinwell et al., 2013). Surely, by today's standards, caffeine would perfectly qualify as a genuine ED at dose levels relevant to human exposure.

Today, a huge number of cellular and nuclear receptors and subreceptors are known, many of which respond to hormones. For example, the International Union on Basic and Clinical Pharmacology (IUPHAR) has established a database that includes 3500 ligand *classes* (IUPHAR, 2013). Taking into account the ubiquitous presence of hormones in the organism and their role in its regulation on a cellular level (OECD DRP, 2012; Godman and Gilman, 2011), one could ask a provocative question: would not most substances, when administered at doses sufficiently high to affect a target organ ultimately display an endocrine disrupting potential somewhere in the organism? For example, it is known that table salt intake and subsequent changes in sodium plasma concentrations may affect renal hormones with subsequent effect on blood pressure (Isozaki et al., 1995). Table salt may also interact with the activity of oral contraceptives (Pechère-Bertschi et al., 2003). Water intake may affect human adrenal, renal and hepatic hormones, such as aldosterone, renin or angiotensin levels (Testai et al., 2013). Would this make table salt or water potential EDs? In other words, the majority of known substances could possibly be classified as potential EDs when tested in depth. Yet, would such a classification contribute to a human health benefit?

Paradoxically, our society and its regulators appear to be quite tolerant towards potent substances that are confirmed EDs and are taken orally by a large number of humans. These examples show how the focus on purported man-made, endocrine disrupting chemicals (EDCs) distracts from potential health risks of human exposure to other substances with far greater potential for hormonal activity. Perhaps the entire issue of purported health risks of *chemical* EDs is just another version of the trivial dichotomy *natural is good* versus *man-made is bad*.

Finally, when reflecting on the presence of all these man-made, albeit hypothetical, EDCs in the environment, why should EDCs primarily affect the male sex, such as a potential decline in *male* fertility, a hypothetical increase in the incidence of hypospadias and cryptorchidism in *male* babies, increase in the incidence of *male* testicular cancers, a reduced penis length in *male* alligators (Guilette et al., 1996), or cause feminisation of *male* fish? Why should females be less affected? Are females less susceptible and, if so, why? On the other hand, could there be a *male* sex-bias concerning these imaginary risks? Recently, it has been claimed that boys from mothers with higher phthalate levels in their urine showed a reduced tendency for *masculine* play behaviour, such a preference of *toys* over *masculine* play. Interestingly, girls seemed unaffected by maternal phthalate levels (Swan et al., 2009). True or not, mothers may rather be relieved about reduced *masculine* play behaviour of their sons. However, some fathers would probably be more concerned about such data: *my boy showing un-masculine*, i.e. *feminised* play

behaviour? Would not terms, such as *feminisation of males, reduced penis length and size* or a *declining male fertility* mainly strike *male* imaginations and sensitivities – and, after all, most researchers in the field of EDCs and, more importantly, politicians handing our research funds are *men*. Could this be a coincidence? One may wonder whether the entire issue of EDC is more within the competence of Dr. Sigmund Freud than that of toxicology.

Overall, it appears weird how the supporters of the ED hypothesis appear to be mesmerised by the idea that a handful of weakly acting chemicals that involve produce little or negligible human exposure must be somehow responsible for a range of hypothetical adverse effects on human reproduction or other human health problems. About 150 years ago, Alexandre Dumas coined the phrase *cherchez la femme!* - meaning that, in every human crime, there is always a woman involved who plays a key role [Translation of the complete phrase: *There is a woman in every case; as soon as they bring me a report, I say, 'Look for the woman!'* (Dumas, 1871)]. Today, this view may strike us as somewhat naïve and sexist. In contrast, today's popular belief rather appears to be: *cherchez le produit chimique!* (Translation: *look for the chemical!*) - expressing the belief that, in every human health problem, man-made, synthetic chemicals must play a key role. Would this not be equally naïve?

Conflict of interest

The opinions forwarded in the article represent personal opinions of the authors and are proposed with the aim to improve the safety assessment of personal care products. The authors received no support or compensation for writing the article. They therefore declare no conflict of interest.

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