RESEARCH ARTICLE

A Historical Perspective on Endocrine Disruption as an Emerging Multifaceted Medical Problem

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Conflict of interest: None.
Source of funding: None

Abstract:

This historical perspective outlines how advances in endocrinology are uncovering mechanisms by which environmental chemicals may interfere in the synthesis and actions of hormones. Such chemicals have been termed endocrine disrupting chemicals (EDCs). Advances in understanding of receptor-mediated genomic and non-genomic actions of hormones have demonstrated the ability of EDCs to compete with hormone for binding to receptors in target cells and thereby to either mimic or antagonise hormone action. Advances in development of a range of assays for identifying endocrine activity of environmental chemicals have led to an appreciation of the wide distribution of EDCs to which the human population are now exposed in air, water, food and consumer products. Technological advances for measuring EDC concentrations in human tissues have demonstrated their widespread presence in human urine, blood, milk, adipose tissue, placenta and breast. Advances in understanding of the wide-ranging physiological effects of hormones and underlying defects in endocrine disorders have begun to reveal how EDCs may provide a new dimension to endocrine dysfunction. Evidence is accumulating for a role of EDCs in many male and female reproductive disorders, thyroid and adrenal disorders, immune system dysfunction and metabolic disorders including obesity, diabetes and cardiovascular disease. Deregulation of hormonally controlled cell growth may contribute to development of endocrine cancers. Adverse effects of EDCs on developmental processes, including neurodevelopment, have implications for child health and for appearance of disorders not only in later adult life but also into future generations.

Keywords: Endocrine disruption, endocrine-disrupting chemicals, endocrine disrupters.

1. Introduction

> ***"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"*** [1]**.**

In almost every area of biology, advances in basic science are needed before disease or aberrant mechanisms can be unravelled. Accordingly, advances in understanding of endocrinology have also been a prerequisite for understanding how environmental chemicals are causing endocrine disruption. Over the past century. as man-made chemicals have been released into the ecosystem from agricultural and industrial activities of man, many of these chemicals have been found to interfere in the synthesis and actions of hormones and such chemicals have been termed endocrine disrupting chemicals (EDCs) [2].

2. Early warnings of endocrine disruption came from declines in animal populations

Although endocrine disruption has only received high profile scientific and public attention over the past 30 years, the ability of environmental chemicals to disrupt hormone actions has become increasingly evident from the loss of fertility of farm animals and reproductive failure in wildlife populations over the past century (Figure 1). In the 1920s, pig farmers in the United States documented a lack of fertility in swine herds when fed mouldy grain [3], and further reports in the 1940s from sheep farmers in Western Australia documented infertility in their sheep after grazing on specific fields of clover [4]. Times were tough for these farmers and loss of fertility in their animals meant loss of livelihoods. More recent research has shown that the underlying mechanisms resulted from consumption of estrogenic compounds contained within the mould (mycoestrogens) [5] or plant material (phytoestrogens) [6], which were disrupting fertility through their potent estrogenic activity.

Over the following decades, endocrine-disrupting properties were reported widely in wildlife living in water, in air and on land after exposure to agricultural and industrial chemicals which were being used increasingly liberally and becoming more widespread in the ecosystem (Figure 1). Effects of the pesticide dichlorodiphenyltrichloroethane (DDT) and its metabolites were reported in birds [7] and mammals [8,9], which coincided with controversial warnings of more widespread consequences of chemical pollution for wildlife populations from organochlorine compounds. Publication in 1962 of the book "Silent Spring" by Rachel Carson [10] was a significant landmark in generating public awareness of endocrine disruption as a widespread phenomenon causing loss of wildlife populations after the liberal agricultural use of pesticides and herbicides and contamination of the land with other organochlorine compounds. The impact of this book was such that it led to international environmental organisations to champion the issues raised. However, a follow-on book entitled "Our Stolen Future" published by Colborn and colleagues at the Silent Spring Institute in the United States in 1996 [11] gave even more serious warnings and demonstrated the inadequacy of measures taken in the intervening years.

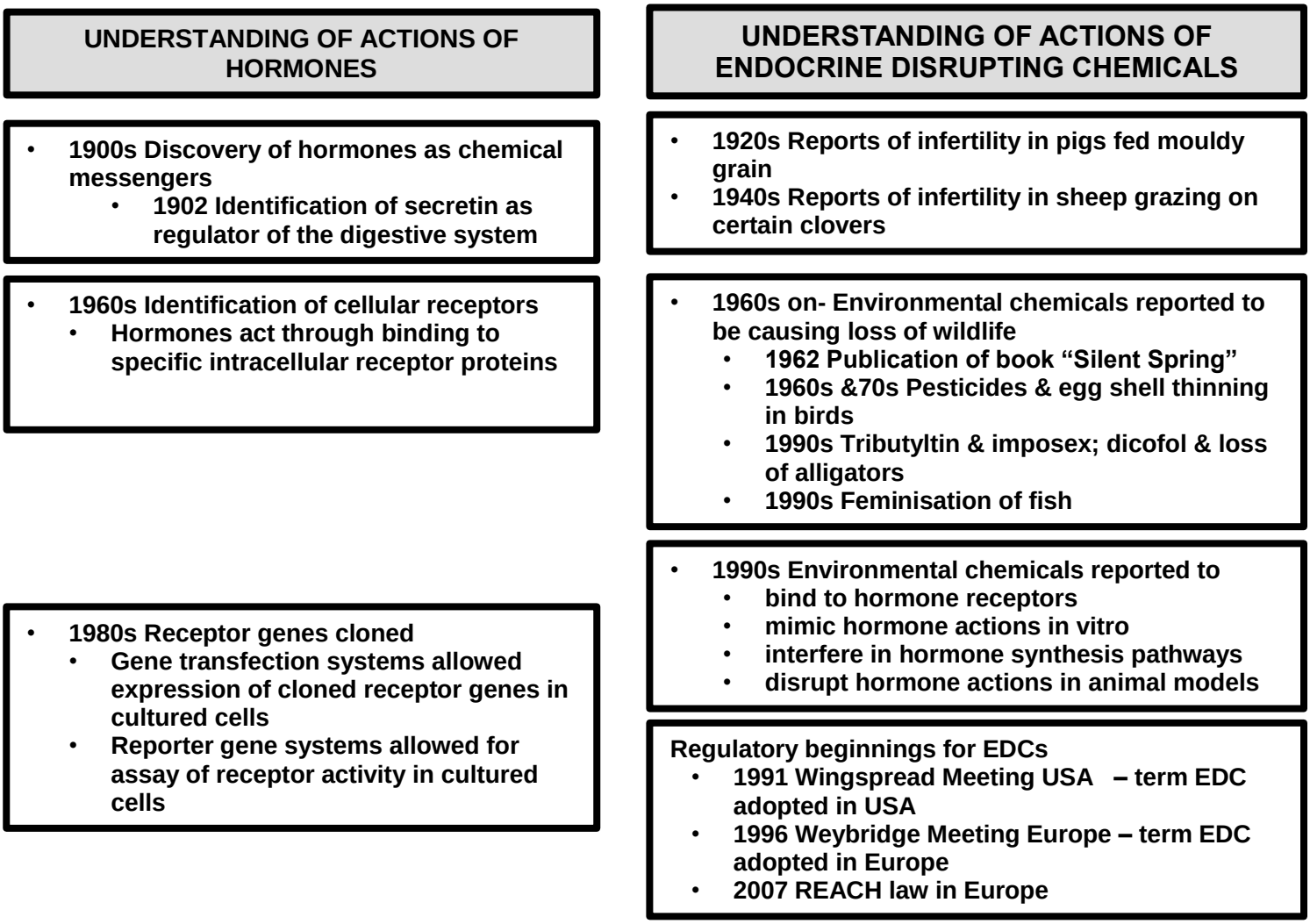

Figure 1. Correlation between historical landmarks in advances in endocrinology and historical landmarks in the recognition of endocrine disruption.

Early breakthrough in identifying specific causes of environmentally-driven endocrine disruption (Figure 1) began with demonstration of the loss of populations due to imposex in bivalves and gastropods in harbour waters from use of tributyltin in antifouling paints applied to the underside of boats [12,13]. Sudden loss of the alligator population in Lake Apopka in Florida, United States was shown to result from a specific spill of dicofol into the lake [14]. In the United Kingdom, feminisation of male fish was reported downstream of sewage effluent works and linked to inadequate removal of estrogenic compounds in the released water [15,16]. Loss of bird populations has been extensively documented as resulting from eggshell thinning associated with pesticide exposures [17-19]. The strongest evidence of causality has been through the demonstration of the reversal of problems following reduction in chemical exposure [20]. The question much debated is now as to whether adverse effects might also occur in the human population in response to the same chemicals and therefore whether the endocrine disrupting effects reported in wildlife might be a warning of impending consequences to human health. Endocrine mechanisms across species show remarkable similarities, but the

scope needed for invoking any precautionary principle for the human population will be an immense challenge for endocrinology to provide sufficient causative evidence to motivate political will.

3. Early warnings of endocrine disruption in humans have come from the legacy of diethylstilboestrol

The ability to synthesise hormones was a major advance in endocrinology. This began in the 1930s with the synthesis of a range of chemicals with oestrogenic activity including diethylstilboestrol [21] and led during subsequent decades to chemical synthesis of many other hormones and a realisation of the pharmaceutical potential of such compounds. Synthetic oestrogens and progestins were used to control female reproductive functions through development of the contraceptive pill in the 1960s [22], and then later hormone replacement therapy [23]. Synthetic glucocorticoids were developed as anti-inflammatory agents [24] and more recently the use of dexamethasone to reduce inflammation during covid-19 infections has been a major life-saver worldwide [25]. Antioestrogens and antiandrogens provide effective targeted therapies to reduce tumour growth in cancer, as do also the aromatase inhibitors for breast cancer [26].

However, it was the synthesis of diethylstilboestrol (DES) in 1938 [21] and its subsequent use to prevent miscarriage in the first trimester of pregnancy [27] that has revealed the strongest warnings of adversity from endocrine disruption in the human population, and in particular the consequences of administering such a potent synthetic oestrogen to women during the vulnerable stages of pregnancy. Several million women were prescribed DES between 1940 and 1971 until it was reported to have caused a rare vaginal cancer in some daughters born to the women who had taken DES during pregnancy [28]. From 1971, prescription ceased but the legacy lives on. Long-term follow-up studies have shown that in utero exposure to DES is associated with a lifelong increased risk of a range of adverse reproductive health outcomes for both daughters and sons [29]. Furthermore, the adverse consequences are also now being reported as passing on to the following generations of daughters [30] and sons [31] born to the DES-exposed women without any further exposure. These reports demonstrate that exposure to this potent synthetic oestrogen in the foetal life of a woman can have consequences not only for her reproductive health in later adult life but also for that of her children without the need for any further exposure. Furthermore, the effects on the DES-exposed sons show that it is not only the female line but also the male line which can be vulnerable. However, the fact that not every DES-exposed daughter or son had adverse symptoms emphasizes the diversity in response between individuals and shows the difficulty in identifying causative agents of hormone disruption where variations in susceptibility can influence outcomes.

4. Advances in molecular endocrinology reveal the ubiquity of endocrine disruptors

The ability to disrupt hormone actions has been known about since ancient times, not least in the context of the use of castration to change serving males into eunuchs, but an appreciation of hormones as specific identifiable chemical messengers began only in 1902 with the identification of secretin in the regulation of the digestive system [32] (Figure 1). However, it was in the late 1950s and early 1960s that the molecular actions of hormones started to be understood and that their mode of action was shown to involve binding to specific cellular receptor proteins, most notably from the pioneering work of Elwood Jensen on the oestrogen receptor [33]. Developments in protein chemistry allowed

estrogen receptor protein to be purified [33], developments in chemical synthesis of oestrogenic compounds [21] and improvements in methods for radiolabelling synthetic hormones allowed specific binding of hormone to receptor protein to be demonstrated [33]. Subsequent work showed that estrogen binds to receptor in the cytoplasm of a cell and that the complex then moves to the nucleus [34-36] leading to induction of specific mRNAs [37]. Further purification of estrogen receptor protein led to development of antibodies [38,39] which ultimately allowed the cloning of estrogen receptor cDNA in the mid 1980s [40]. Analogous pathways of discovery were followed for other steroid receptors and other members of the nuclear receptor superfamily [41].

These advances in molecular endocrinology allowed for the development of assays showing that hormone-receptor complexes could bind to DNA and function as transcription factors capable of regulating gene expression [41] (Figure 1). It was these same assays which were then also used to test the ability of different ligands to act as agonists or antagonists of specific receptors and hence allowed EDCs to be tested for their ability to disrupt specific receptor systems and specific receptor-mediated gene expression profiles [2] (Figure 1). Initially, most reports were of estrogen- or androgen-disrupting activity because these were the assay systems most widely available, but as time has progressed, disruption through many other receptor systems have been reported as the assays have become available [2]. Some environmental chemicals, particularly organochlorine compounds, were shown to bind to another nuclear receptor termed the aryl hydrocarbon receptor causing alterations to expression of cytochrome P450 genes [42]. Since some of the P450 genes encode enzymes acting in the synthesis and/or metabolism of hormones, the mechanism of action of EDCs became understood as involving disruption to synthesis and metabolism of hormones as well as effects through altering gene expression [42]. Now, more recently, non-genomic mechanisms of action of hormones are being unravelled, which may explain the rapid actions of hormones, and as assays have developed, so EDCs have been shown to interfere also in cell-signaling phosphorylation cascades [43,44]. Over recent years, EDCs have also been shown to interfere in receptor-mediated epigenetic pathways through altering enzymes involved in DNA methylation and histone acetylation [45].

Advances in development of such a range of assays for endocrine activity of compounds [2] have led to an understanding of the wide range of environmental chemicals distributed across global ecosystems which can act as endocrine disruptors, and the extent to which the human population is exposed to EDCs on a daily basis from contaminants in drinking water [2], air [46], food [2], or consumer products [2] as summarised in Figure 2.

5. Development of analytical endocrinology has shown the wide uptake of endocrine disruptors into human tissues

Assessment of the ability of environmental EDCs to enter human tissues from the many exposure routes (Figure 2) has been a priority area for investigation and this has been dependent on technological advances in detection limits for measuring chemicals in human body tissues using chromatographic separation methods and mass spectrometry. Although most EDCs bind more weakly to hormone receptors than do the physiological hormones [47], their presence in tissues is still significant at micromolar levels and even at nanomolar levels and especially when considering additive effects of mixtures of chemicals [48-51]. EDCs have now been detected globally in human urine, blood and milk which are the

most accessible sampling methods, but more specialised tissue samples such as placenta, adipose tissue and breast tissue have also been reported as containing EDCs [2].

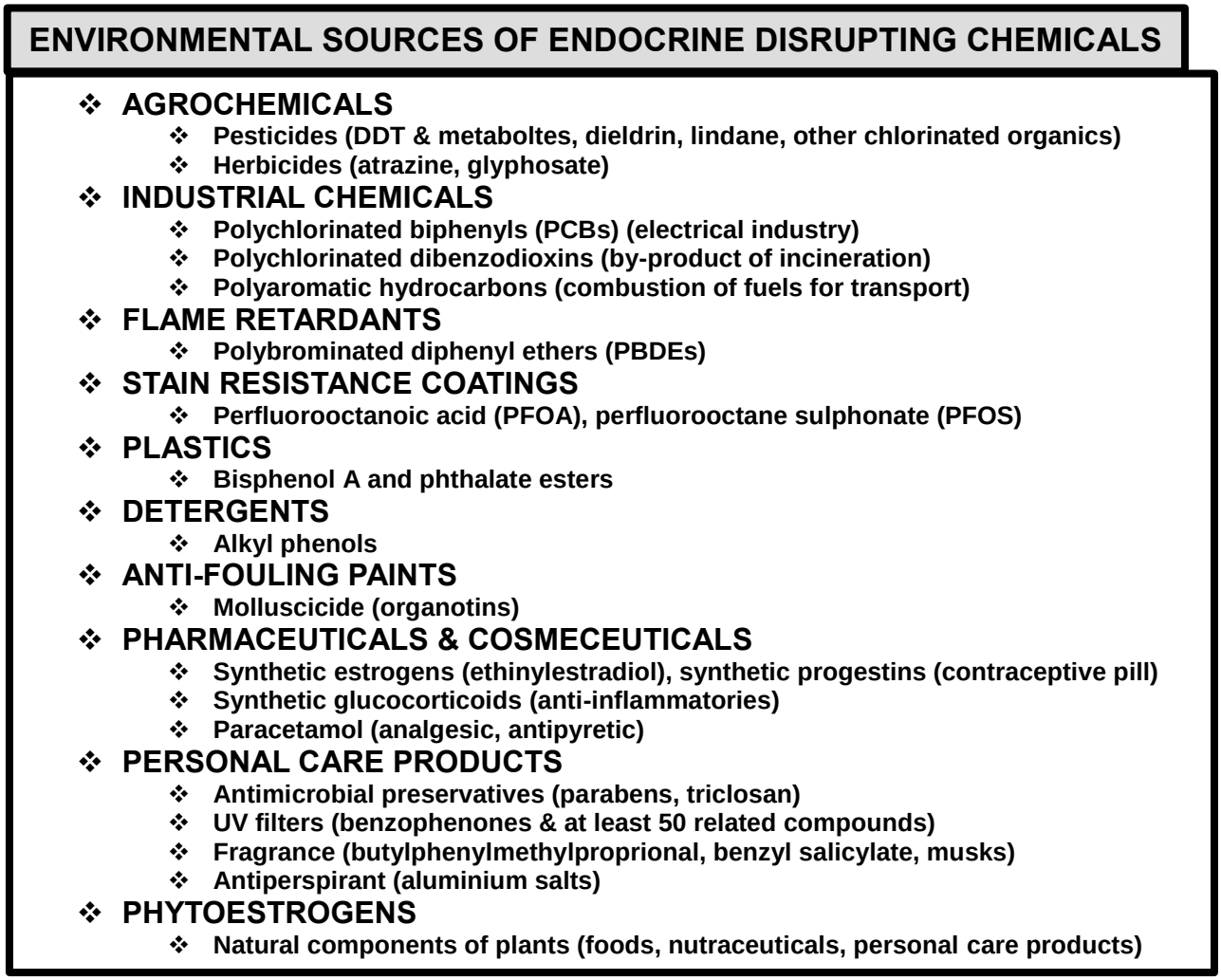

Figure 2. Advances in molecular endocrinology have enabled development of assay systems which demonstrate the wide range of environmental chemicals with endocrine-disrupting activity. Endocrine disrupters are found in diet, in air and in many consumer products. Some are naturally occurring (such as the plant phytoestrogens) but the majority are man-made chemicals released into the ecosystem by human activities (for detailed references see reference 2).

Over the past 20 years, establishment of the National Health and Nutrition Examination Survey (NHANES) in the United States has demonstrated the widespread contamination of the US population. Under the NHANES project, many EDCs have been measured widely in human urine including phthalate esters (used in plastics) [52], bisphenol A (BPA) (used in plastics) [53], 4-tertiary-octylphenol (used to make resins and surfactants) [53], triclosan (used as an antimicrobial agent) [54], parabens (used as preservatives) [55] and benzophenone-3 (used as an ultraviolet (UV) filter) [56]. Although the presence of EDCs in human tissues cannot be taken alone to imply any adverse consequences, demonstration of their presence is a prerequisite for any adverse actions and development of increasingly sensitive methods for their detection in

human tissues has brought an awareness of their entry into human tissues which was previously not considered to be possible.

6. Advances in medical endocrinology reveal consequences of EDC exposure for human health

Over the past century, substantial advances have been made in understanding of the wide-ranging physiological effects of hormones and in the underlying defects in endocrine disorders [57,58]. The ability of environmental chemicals to cause endocrine disruption provides a new dimension to endocrine dysfunction [2]. Evidence is now accumulating for a causative role of EDCs in many male and female reproductive disorders, thyroid and adrenal disorders, developmental disorders, immune system dysfunction and metabolic disorders, including obesity, diabetes and cardiovascular disease [2] (Figure 3). Deregulation of hormonally controlled cell growth may contribute to the development of endocrine cancers [2]. Adverse effects of EDCs on developmental processes, including neurodevelopment, have implications for child health and development of disorders later in adult life [2] (Figure 3).

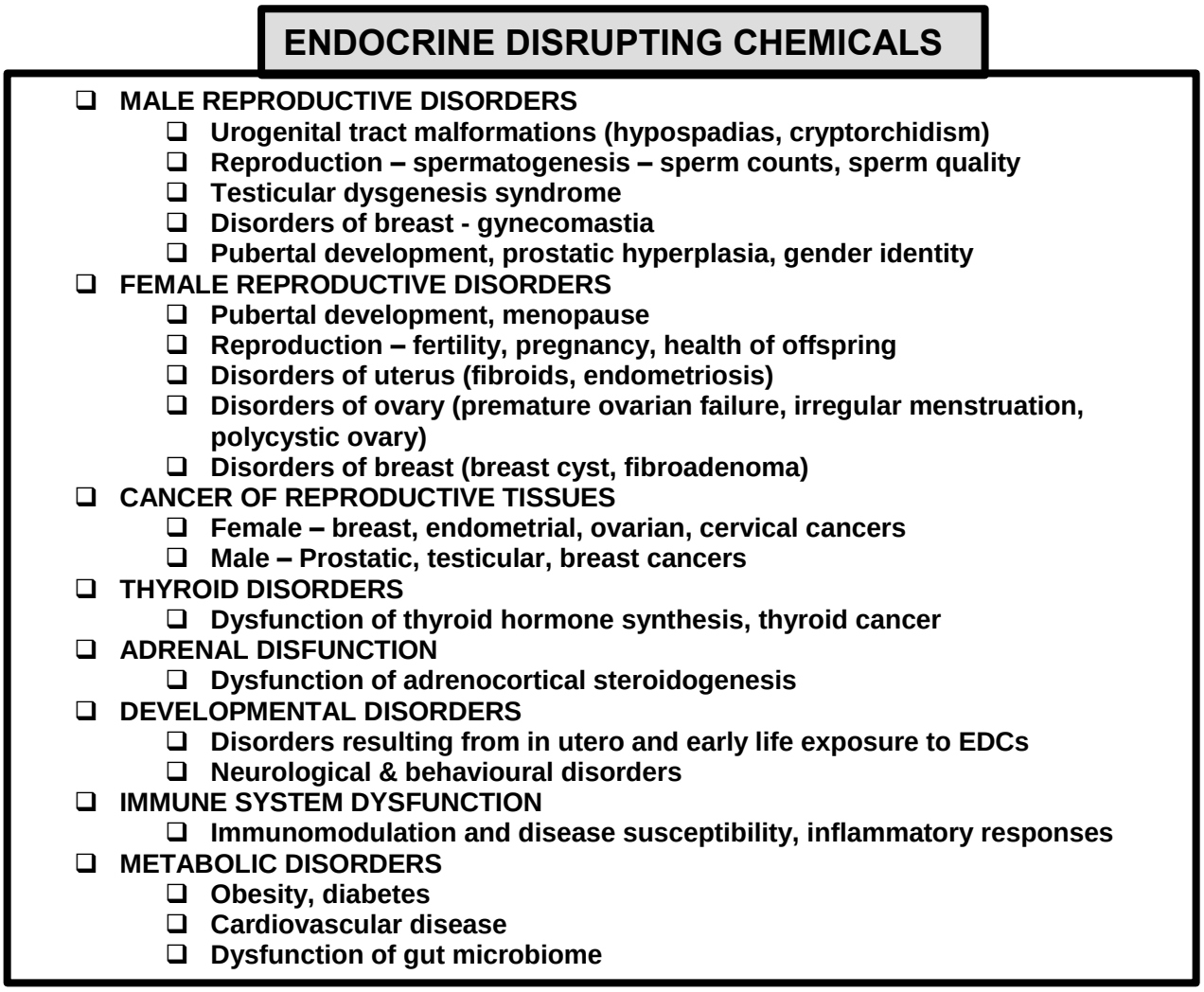

Figure 3. Advances in medical endocrinology are showing the many human endocrine disorders which result from exposure to endocrine-disrupting chemicals (for more details see reference 2).

The effects of exposure to DES during human development in utero gave early warning that humans could be vulnerable to endocrine disruption with long-lasting consequences [28-31]. However, a new focus on human health opened up in the early 1990s with reports of declining sperm quality in men living in Denmark compared with men living in Finland, its less industrialised neighbour, and this was proposed to relate to exposure to pollutant chemicals [59]. The decline in sperm quality was associated with incidence of hypospadias, cryptorchidism and testicular cancer defining a new testicular dysgenesis syndrome [60] and opening the door to wider discussions concerning environmental chemicals as causative agents in other increasingly reported not only male but also female reproductive problems [2].

Another new focus on human health was also opened in the 1990s when David Barker proposed a link between aberrant foetal development and adult disease [61]. With the unfolding legacy of reproductive consequences following in utero exposure to DES [28-31], wider discussions were initiated on the potential for other EDCs to impact on foetal development with long-lasting consequences for adult health [62-64]. When some EDCs were demonstrated to possess obesogenic properties, concerns were raised for the potential for exposure to such chemicals in foetal life to be linked with the epidemic of obesity [65]. Given the evidence that breast cancer is an endocrine-regulated cancer with origins often in early life, questions need to be asked about the potential for EDC exposure to be causative in the rising incidence observed now worldwide [66] and such questions could be relevantly expanded to other endocrine-related cancers [2].

The demonstration that so many environmental chemicals can act by disrupting hormone action has brought a whole new dimension to medical toxicology, because by acting through receptor-mediated processes, adverse effects can occur at much lower concentrations and in a much more targeted manner than would occur for classical nonspecific toxicity. Furthermore, the non-monotonic actions of hormone receptor-mediated mechanisms have also challenged the basic premise of toxicology that "the dose makes the poison", a concept first introduced by the Swiss chemist Paracelsus (1493-1541). Since dose-response curves for receptor-mediated actions may not always be linear [67], with mechanisms at high concentrations often different from those at low concentrations, this challenges classical toxicological responses in which higher doses are expected to give greater toxicity. Unravelling the many ways in which EDCs can disrupt hormone action has occurred alongside advances in endocrinology, and EDCs have been shown to interfere in many different endocrine pathways both as individual chemicals and as complex mixtures [48-51] as outlined in Figure 4 [2]. In assessing the outcomes of exposure to EDCs, it must be taken into account that there will be differences in response between different tissues and different individuals [2]. Tissue responses will depend not only on relative receptor concentrations and cellular environment of the target cells but also on the wider microenvironment within the tissue [68]. Variations in responses of different individuals will depend on a complexity of interactions between the individual genetic background and lifestyle factors. In this context, it must be considered that EDCs can compromise human health, even if they do not do so for every person [2].

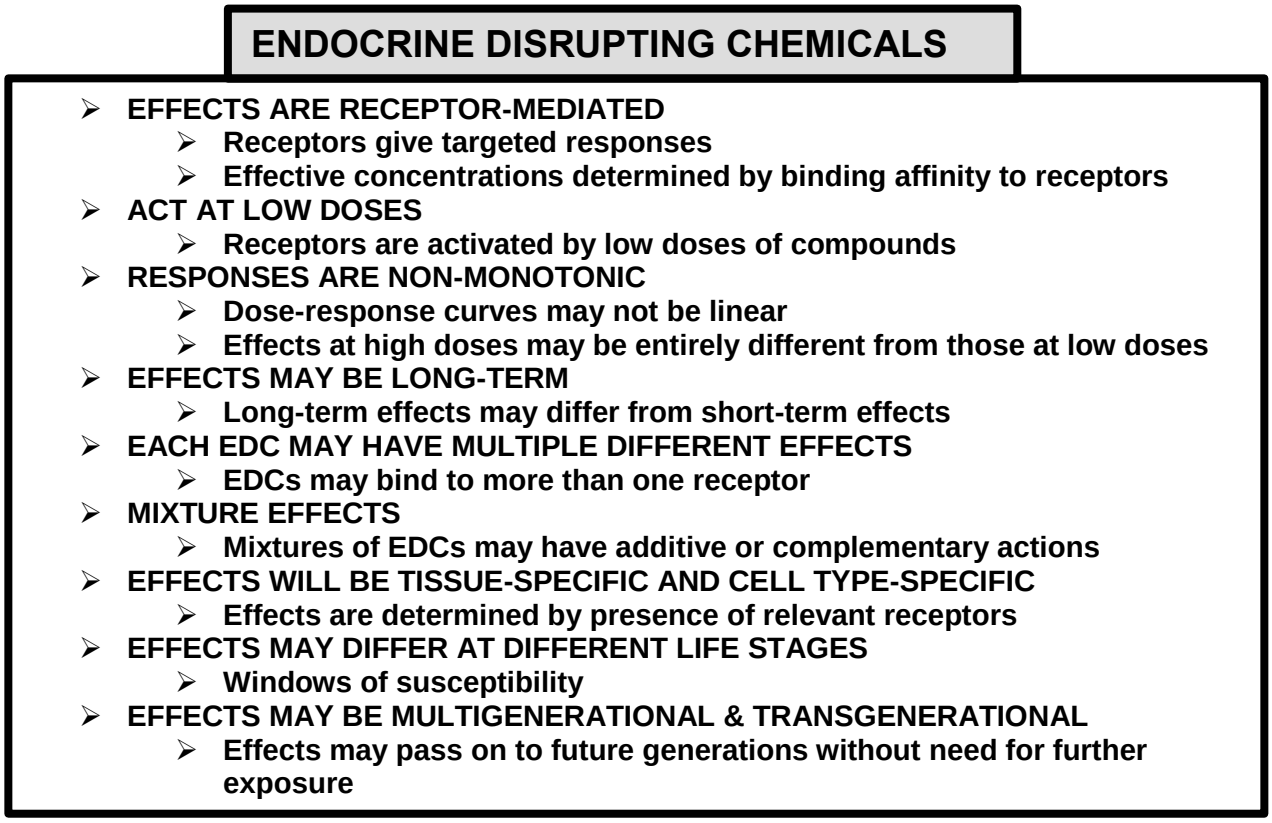

Figure 4. Mechanisms of action of endocrine-disrupting chemicals which characterise them as differing from non-specific toxic compounds. Their ability to bind to hormone receptors allows for them to act at much lower doses and in a more targeted manner than non-specific compounds (for more details see reference 2).

7. Concluding comments

Modern life appears to be making it inevitable that the human population will continue to be exposed on a daily basis to complex mixtures of EDCs from air, water, food and consumer products. Although concentrations of individual chemicals may be low, uptake into human tissues and bioaccumulation of lipophilic EDCs ensures that chemicals, which did not even exist 100 years ago, are now ubiquitously present in the human body and outcomes will depend not only on exposures but also on individual susceptibilities.

Over recent decades, there have been many discussion meetings about how regulatory measures might be taken to reduce exposure to EDCs. One of the most significant early scientific meetings on EDCs was the World Wildlife Fund Wingspread Conference in Wisconsin in the USA in 1991, which was where the term "endocrine disrupter" was first proposed [69]. Europe has played a significant role in championing the regulation of EDCs. The first European meeting on EDCs was held in 1996 in Weybridge to discuss the implications of findings [70], and a further meeting 15 years later in Finland reported on progress made from European-funded projects [71]. EDCs have been addressed in several acts of European law including The Water Framework Directive (European Parliament 2000), The European Registration, Evaluation, Authorisation and Restriction of CHemicals (REACH) legislation (European Parliament 2006), The Plant Protection Products Regulation (PPPR) (European

Parliament 2009), The Cosmetics Regulation (European Parliament 2009) and the Biocidal Products Regulation (European Parliament 2012) [72].

The ability of different combinations of EDCs to cause similar clinical outcomes provides a very real problem for any regulatory actions or preventative measures. Some adverse effects such as gynecomastia caused by exposure to dermal estrogenic EDC exposures in men can be clearly reversible upon ceasing exposure, thus allowing for the causative agents to be identified [73-77]. However, the long-term, multifactorial nature of other adverse health outcomes such as cancer is much more difficult [2]. Nevertheless, research must move to a position of being able to advise on how to avoid the many adverse clinical outcomes which are on the rise, not least in relation to EDC exposures of mothers during pregnancy and of early life exposures in children. Even if an EDC is only part of a mixture and can be substituted by another EDC acting via a similar mechanism, it makes it no less problematic. Even if an EDC is not always present at higher concentrations in blood or tissue of an individual affected by an adverse endocrine health outcome, it makes it no less culpable in those individuals where it is present or present as part of a mixture. All this leads to a conclusion that regulating individual chemicals is not enough and that EDC exposure needs to be reduced "across the board". The human body is remarkably tolerant of toxic insult but for every individual there is likely to be a point where the body can no longer cope with more. This is expressed very well in the idiom "the straw that breaks the camel's back", which describes a seemingly minor or routine action that causes an unpredictably large and sudden reaction because of the cumulative effect of small actions. This concept has been well known in mathematics as "Catastrophe Theory", a theory developed in the 1960s by the French mathematician René Thom to make predictions of gradually changing processes which may lead to sudden changes [78]. The parallels with increasing exposures to EDCs in modern life are stark, and this maybe serves to remind us, as endocrinologists, of our need to be mindful of advances in all areas of endeavour and their applicability to our own field of interest.

References

1. World Health Organisation, International Labour Organisation, and United Nations Environment Programme. Global assessment of the state-of-the-science of endocrine disruptors, (editors T. Damstra, S. Barlow, A. Bergman, R. Kavlok, G. VanderKraak) (WHO/PCS/EDC/02.2) (2002) Available at: https://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/ [accessed 04 May 2021].
2. Darbre PDD (editor). Endocrine Disruption and Human Health, Elsevier, 2015 (second edition pending autumn 2021).
3. McNutt SH, Purwin P, Murray C. Vulvo-vaginitis in swine: preliminary report. *J Am Vet Med Assoc*. 1928; 73: 484.
4. Bennets H, Underwood EJ, Shier FL. A specific breeding problem of sheep on subterranean clover pasture in Western Australia. *Aust Vet J*. 1946; 22: 2-12.
5. Bennett JW, Klich M. Mycotoxins. *Clin Microbiol Rev*. 2003; 16: 497-516.
6. Woods, H.F. (chairman), Phytoestrogens and health. Crown copyright UK, 2003.
7. Burlington H, Linderman VF. Effect of DDT on testes and secondary sex characteristics of white leghorn cockerels. *Proc Soc Exp Biol Med*. 1950; 74: 48–51.
8. Bitman J, Cecil HC, Harris SJ, Fries GF. Estrogenic activity of o,p′-DDT in the mammalian uterus and avian oviduct. *Science.* 1968; 162: 371–372.
9. Welch RM, Levin W, Conney AH. Estrogenic action of DDT and its analogs. *Toxicol Appl Pharmacol.* 1969: 4; 358–367.
10. Carson R. Silent Spring. USA: Houghton Mifflin, 1962.
11. Colborn T, Dumanoski D, Myers JP. Our Stolen Future. Dutton, USA.1996.
12. Horiguchi T. Masculinization of female gastropod mollusks induced by organotin compounds, focusing on mechanism of actions of tributyltin and triphenyltin for development of imposex. *Environ Sci.* 2006; 13; 77–87.
13. Matthiessen P. Historical perspective on endocrine disruption in wildlife. *Pure Appl Chem.* 2003; 75: 2197–2206.
14. Guillette LJ, Gunderson MP. Alterations in development of reproductive and endocrine systems of wildlife populations exposed to endocrine-disrupting contaminants. *Reproduction.* 2001; 122; 857–864.
15. Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP. Widespread sexual disruption in wild fish. *Environ Sci Technol*. 1998; 32: 2498–2506.
16. Harries JE, Janbakhsh A, Jobling S, Mattiessen P, Sumpter JP, Tyler CR. Estrogenic potency of effluent from two sewage treatment works in the United Kingdom. *Environ Toxicol Chem.* 1999; 18: 932–937.
17. Ratcliff DA. Decrease in eggshell weight in certain birds of prey. *Nature*. 1967; 215: 208–210.
18. Ratcliff DA. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. *J Appl Ecol*. 1970; 7: 67–115.
19. Lundholm CE. DDE-induced eggshell thinning in birds: effects of p,p′-DDE on the calcium and prostaglandin metabolism of the eggshell gland. *Comp Biochem Physiol Part C Pharmacol Toxicol Endocrinol.* 1997; 118: 113–128.
20. Bergman A, Heindel JJ, Jobling S, Kidd KA, Zoeller RT (editors). The state of the science of endocrine disrupting chemicals—2012. United Nations Environment Programme (UNEP) and

World Health Organisation (WHO), 2013.
21. Dodds EC, Goldberg L, Lawson W, Robinson R. Estrogenic activity of certain synthetic compounds. *Nature*. 1938; 141: 247-248.
22. Stanczyk FZ, Archer DF, Bhavnani BR. Ethinyl estradiol and 17β-estradiol in combined oral contraceptives: pharmacokinetics, pharmacodynamics and risk assessment. *Contraception*. 2013; 87: 706-727.
23. Mattox JH, Shulkman LP. Combined oral hormone replacement therapy formulations. *Am J Obstet Gynecol*. 2001; 185: S38-S46.
24. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids - new mechanisms for old drugs. *New Engl J Med*. 2005; 353: 1711-1723.
25. The Recovery Collaborative Group. Dexamethasone in hospitalized patients with Covid-19. *New Eng J Med*. 2021; 384: 693-704.
26. Lonning, P.E. (editor), Endocrinology and treatment of breast cancer. *Clin Endocrinol Metab*. 2004; 18: 1-130.
27. Smith OW. Diethylstilboestrol in the prevention and treatment of complications of pregnancy. *Am J Obstet Gynecol*. 1948; 56: 821-834.
28. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina: association of maternal stilboestrol therapy with tumor appearance in young women. *New Engl J Med*. 1971; 284: 878-881.
29. Harris RM, Waring RH. Diethylstilboestrol - a long-term legacy. *Maturitas* 2012; 72: 108-112.
30. Titus L, Hatch EE, Drake KM, et al, Reproductive and hormone-related outcomes in women whose mothers were exposed in utero to diethylstilbestrol (DES): A report from the US National Cancer Institute DES Third Generation Study. *Reprod Toxicol*. 2019; 84: 32-38.
31. Kalfa N, Paris F, Soyer-Gobillard MO, Daures JP, Sultan C. Prevalence of hypospadias in grandsons of women exposed to diethylstilbestrol during pregnancy: a multigenerational national cohort study. *Fertility and Sterility*. 2011; 95: 2574-2577.
32. Bayliss WM, Starling EH. The mechanism of pancreatic secretion. *J Physiol*. 1902; 28: 325–353.
33. Moore DD. A conversation with Elwood Jensen. Ann Rev Physiol. 2012; 74: 1-11.
34. Jensen EV, Suzuki T, Kawashima T, Stumpf WE, Jungblut PW, DeSombre ER. A two-step mechanism for the interaction of estradiol with rat uterus. *Proc Natl Acad Sci USA*. 1968; 59: 632–638.
35. Toft D, Gorski J. A receptor molecule for estrogens: isolation from the rat uterus and preliminary characterization. *Proc Natl Acad Sci USA*. 1966; 55: 1574–1581.
36. Gorski J, Toft D, Shyamala G, Smith D, Notides A. Hormone receptors: studies on the interaction of estrogen with the uterus. *Recent Prog Horm Res*. 1968; 24: 45–80.
37. O'Malley BW, Means AR. Female steroid hormones and target cell nuclei. *Science*. 1974; 183: 610–620.
38. Greene GL, Closs LE, Fleming H, DeSombre ER, Jensen EV. Antibodies to estrogen receptor: immunochemical similarity of estrophilin from various mammalian species. *Proc Natl Acad Sci USA*. 1977; 74: 3681–3685.
39. Greene GL, Fitch FW, Jensen EV. Monoclonal antibodies to estrophilin: probes for the study of estrogen receptors. *Proc Natl Acad Sci USA*. 1980; 77: 157–161.

40. Green S, Walter P, Greene G, et al. Cloning of the human oestrogen receptor cDNA. *J Steroid Biochem*. 1986; 24: 77–83.
41. Parker, MG. (editor). Nuclear Hormone Receptors: Molecular Mechanisms, Cellular Functions, Clinical Abnormalities. Academic Press, 1991.
42. Nebert DW. Aryl hydrocarbon receptor (AHR): "pioneer member" of the basic-helix/loop/helix per-Arnt-sim (bHLH/PAS) family of "sensors" of foreign and endogenous signals. *Prog Lipid Res.* 2017; 67: 38–57.
43. Acconcia F, Marino M. The effects of 17β-estradiol in cancer are mediated by estrogen receptor signaling at the plasma membrane. *Front Physiol*. 2011; 2: 1–8.
44. Soltysik K, Czekaj P, Membrane estrogen receptors—is it an alternative way of estrogen action?. *J Physiol Pharmacol*. 2013; 64: 129–142.
45. Alavian-Ghavanini A, Ruegg J. Understanding Epigenetic Effects of Endocrine Disrupting Chemicals: From Mechanisms to Novel Test Methods. *Basic Clin Pharmacol Toxicol.* 2018; 122: 38-45.
46. Darbre PD. Overview of air pollution and endocrine disorders. *Int J Gen Med.* 2018; 11: 191-207.
47. Blair RM, Fang H, Branham WS, Hass BS, Dial SL, Moland CL. The estrogen receptor binding affinities of 188 natural and xenochemicals: structural diversity of ligands. *Toxicol Sci.* 2000; 54: 138–153.
48. Rajapakse N, Silva E, Kortenkamp A. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. *Environ Health Perspect*. 2002; 110: 917–921.
49. Silva E, Rajapakse N, Kortenkamp A. Something from "nothing"—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. *Environ Sci Technol*. 2002; 36: 1751–1756.
50. Kortenkamp A. Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals. *Environ Health Perspect*. 2007; 115 (Suppl. 1): 98–105.
51. Charles AK, Darbre PD. Combinations of parabens at concentrations measured in human breast tissue can increase proliferation of MCF-7 human breast cancer cells. *J Appl Toxicol*. 2013; 33: 390–398.
52. Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999–2000. *Environ Health Perspect*. 2004; 112: 331–338.
53. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. *Environ Health Perspect*. 2008; 116: 39–44.
54. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Urinary concentrations of triclosan in the U.S. population: 2003–2004. *Environ Health Perspect*. 2008; 116: 303–307.
55. Calafat AM, Ye X, Wong LY, Bishop AM, Needham LL. Urinary concentrations of four parabens in the U.S. population: NHANES 2005–2006. *Environ Health Perspect*. 2010; 118: 679–685.
56. Calafat AM, Wong LY, Ye X, Reidy JA, Needham LL. Concentrations of the sunscreen agent benzophenone-3 in residents of the United States: National Health and Nutrition Examination Survey 2003–2004. *Environ Health Perspect*. 2008; 116: 893–897.

57. Tata JR. One hundred years of hormones. *EMBO Reports.* 2005; 6: 490-496.
58. Huhtaniemi I (editor). Encyclopaedia of Endocrine Diseases. 2nd edition. Elsevier, 2018. ISBN: 9780128121993
59. Jensen TK, Vierula M, Hjollund NH, et al. Semen quality among Danish and Finnish men attempting to conceive. The Danish first pregnancy planner study team. *Eur J Endocrinol.* 2000; 142: 47–52.
60. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod.* 2001; 16: 972–978.
61. Barker DJP. Fetal origins of coronary heart disease. *Brit Med J.* 1995; 311: 171-174.
62. Schug TT, Janesick A, Blumberg B, Heindl JJ. Endocrine disrupting chemicals and disease susceptibility. *J Steroid Biochem Mole Biol*. 2011; 127: 204-215.
63. Nesan D, Kurrasch DM. Gestational exposure to common endocrine disrupting chemicals and their impact on neurodevelopment and behavior. *Ann Rev Physiol*. 2020; 82: 177-202.
64. Iguchi T, Sato T, Nakajima T, Miyagawa S, Takasugi N. New frontiers of developmental endocrinology opened by researchers connecting irreversible effects of sex hormones on developing organs. *Differentiation* (2020) (EPubaheadofprint).
65. Darbre PD. Endocrine disruptors and obesity. *Curr Obes Rep*. 2017; 6: 18-27.
66. Darbre P. EDCs and breast cancer cells. *Adv Pharmacol*. 2021; 92 (in press)
67. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Lee DH. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev*. 2012; 33: 378–455.
68. Burks H, Pashos N, Martin E, McLachlan J, Bunnell B, Burow M. Endocrine disruptors and the tumor microenvironment: a new paradigm in breast cancer biology. *Mol Cell Endocrinol*. 2017; 457: 13-19.
69. Colborn T, Clement C. Chemically-induced alterations in sexual and functional development: the wildlife/human connection. Princeton, NJ: Princeton Scientific Publishing Co Inc; 1992.
70. Report of the Proceedings of the European workshop on the impact of endocrine disrupters on human health and wildlife. Weybridge, UK. Report EUR17549 of the Environment and Climate Change Research Programme of DGXII of the European Commission. 1996.
71. European Environment Agency (EEA). The impacts of endocrine disrupters on wildlife, people and their environments. The Weybridge+15 (1996-2011) report. Publications Office of the European Union, Luxemburg, 2012. ISBN 978-92-9213-307-B.
72. Slama R, Bourguignon JP, Demeneix B, et al. Scientific issues relevant to setting regulatory criteria to identify endocrine-disrupting substances in the European Union. *Environ Health Perspect*. 2016; 124: 1497-1503.
73. Edidin DV, Levitsky LL. Prepubertal gynecomastia associated with estrogen-containing hair cream. *Am J Dis Child*. 1982; 136: 587–588.
74. Finkelstein JS, McCully WF, MacLaughlin DT, Godine JE, Crowley WF. The Mortician's Mystery. *New Engl J Med*. 1988; 318: 961–965.
75. Bhat N, Rosato EF, Gupta PK. Gynecomastia in a mortician. A case report. *Acta Cytol*. 1990; 34: 31–34.

76. Felner EI, White PC. Prepubertal gynecomastia: indirect exposure to estrogen cream. *Pediatrics.* 2000; 105 (e55): 1–3.
77. Henley DV, Lipson N, Korach KS, Bloch CA. Prepubertal gynecomastia linked to lavender and tea tree oils. *New Engl J Med*. 2007; 356: 479–485.
78. Thom R. Structural Stability and Morphogenesis: An Outline of a General Theory of Models. CRC Press (2018) (available online through search of the title) – originally published in French in 1972 by WA. Benjamin, Inc.

