Clinical Pharmacodynamics of Endocrine Disruptors: A Historic Perspective

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Abstract

In this review endocrine disruptors (EDCs) are viewed from a historic perspective. A lot of them are among us for 100 years or even more. Nevertheless research in this area is only a few decades old with a lot of conflicting data. Possible causal relationships between EDCs exposure and adverse effects on human health are dependant on the critical window between exposure in utero and early childhood. This critical window can be 20 years or more. As history predicts definite conclusions can only be drawn after replacing or eliminating some EDCs by law. It will take another few decades to observe the effect of these necessary measures.

Keywords
Endocrine disruptors; Pharmacodynamics; Phthalates; Bisphenol A; Sex hormones; Breast cancer miscarriages; Birth weight; Obesity; Cardiovascular disease; Childhood asthma

Introduction

Endocrine-disrupting chemicals or EDCs are synthetic or naturally occurring compounds that can interfere or mimic the body’s hormones. EDCs such as flame retardants, phthalates and bisphenol A are known for their potential effects on reproductive, neurological and immune functions.

Animal studies also suggest that early life exposure to some EDCs can cause weight gain in later life. These are called “obesogens”. The history of EDCs starts in 1958 when the endocrinologist Roy Hertz proposed that certain chemicals in feed diets could find their way into the human body and mimic hormone activity [1].

Effects of EDCs on early development in wildlife and in humans are often irreversible and may not become evident until later in life. At the moment 87,000 chemicals are screened by the EPA (Environmental Protection Agency) for their possible endocrine disrupting effect. EDCs can be classified by their mechanism of action interfering with the different or same target organs as the natural hormone by altering its synthesis, metabolism, binding and cellular action. Pharmacodynamics studies the ligand (drug)-receptor binding which shows similarities with the natural hormone-receptor binding. In this case the drug or ligands are the EDCs. This review will focus on the clinical pharmacodynamics of endocrine disruptors in a historic perspective. In the early 1970s the dramatic health impacts of diethylstilbestrol (DES), an estrogen based drug that was thought to prevent miscarriages, introduced the possibility of hormone disruption as a threat to human health and development, that sparked intensive study of estrogens in the environment. Since the second part of the last century the phytoestrogens have been studied most widely.

Phytoestogens-Natural Disruptors

Phytoestrogens are dietary plant derived estrogen-like compounds found in a variety of plant foods such as beans, seeds and grains, though they are concentrated in soy Foods and flax.

It is important to realize that plants are not the only dietary source of xenoestrogens. There is also estrogen content in animal foods such as meat, dairy, eggs, fish and seafood.

Most research has focused on the plant derived versions and little research has been conducted on animal sources, which is leading to an underestimation of intake [2].

The phytoestrogens are structurally related to 17-beta estradiol as is shown by the chemical structure of the isoflavones genistein and diadzen present in soybeans. The isoflavones belong to a large group of substituted natural phenolic compounds as the coumestans and prenyllavanoinds. Phytoestrogens exert their effect primarily through binding to the estrogen receptor (ER). There are two variants of the ER, the alpha (ER) and beta (ER). Many phytoestrogens and also mycoestrogens have somewhat higher affinity for the beta (ER) compared to the alpha (ER). The ER is a nuclear receptor that is activated by the sex hormone estrogen. Ligands and agonists are the non-selective endogenous estrogens (e.g. estradiol, estrone, estriol, estetrol), natural and conjugated estrogens and synthetic estrogens (eg ethinylestradiol). A selective agonist of the alpha (ER) over the beta (ER) is proplypyrazolestrol (PPT). Mixed agonists are the phytoestrogens (e.g., coumestrol, daidzen, genistein, miroestrol) and the selective estrogen modulators (e.g. tamoxifen, clomifene, raloxifene). The ER antagonists include the non-selective anti-estrogens fulvestrant and ethamoxityphetol. A selective ER antagonist is methylpipiridinepyrazol (MPP). Estrogen insensitivity syndrome is a very rare condition characterized by a defective alpha(ER) that is insensitive to estrogens. Estrogen receptors (ERs) are present in a variety of tissues. Alpha (ER) is most abundant in the uterus and smaller quantities are present in the ovary, testis, skin and gut. High amounts of beta (ER) are present in the ovaries, testes, adrenals and spleen [3]. The ER has important interrelrelationships with the progesterone receptor(PR) system as well with insulin-like growth factor (IGF-1). The harmful or benificial effects of soy have been a dispute for decades. Evidence of isoflavones possibly having harmful effects appeared in the 1940s spurring one of soy’s first controversies. A 1946 in the Australian Veterinary Journal indicated that sheep that grazed on a subterranian isoflavone-rich clover in Western Australia experienced fertility issues and breeding problems [4].

About 80% of the soy currently produced in the United States is used as animal food. By 1959 soybean meal was described an “excellent source of growth promoting” for young farm animals as turkeys [5].
By the early 1970s more studies shed light on the use of soy proteins in baked foods and the functional properties of soy proteins [6-7]. In the 1980s two animal studies suggested that soy diets caused an enlarged pancreas and were associated with the growth of pancreatic cancer in those animals [8,9]. However in 1989 a report by the National Cancer Institute wrote there was no evidence that soybean derived foods had adverse effects on the human pancreas [10]. Rather it was observed that human populations with high levels of soy in the diet had decreased rates of pancreatic cancer [10]. In the 1990s the breast cancer, soy connection came up. In 1991 the first of many studies to suggest that soybean contain potentially anti-carcinogenic benefits appeared [11]. Things changed in 1996 when a study suggested that consuming soy protein might actually stimulate the growth of breast cancer cells [12]. In 2001 another study suggested that soy isoflavones stimulated the growth of human estrogen-dependent breast cancer cells [13].

The confusion about whether soy increases breast cancer risk is related to the fact that the ER has an alpha and a beta receptor, as mentioned before. When a compound binds to the alpha (ER) receptor the cancer risk rises and when it predominantly binds to the beta (ER) the cancer risk decreases. Isoflavones bind to the beta (ER).

A review paper in the journal Cancer suggested an apparent lack of association between soy and cancer [14].

Benefits of soy were also reported. Replacing junk food in your diet by soy can aid in avoiding weight gain as research published in 2004 and 2009 reported [15,16]. Around that time soy protein and isoflavones also gained attention for having a potential role in improving cardiovascular health [17]. In 2008 the AHA (American Heart Association) stated there was not enough evidence to claim a strong link between soy and reduced risk of coronary heart disease [18]. Soy has the antioxidant properties that can lower LDL-cholesterol. It doesn’t increase our good HDL-cholesterol [19]. In the mid 2000s research offered more information on how soy intake may impact thyroid function. The evidence suggests soy can interfere with the body’s ability to absorb L-thyroxine, usually used in the treatment of hypothyroidism. Generally it is recommended to wait four hours before consuming any soy products after taking thyroid hormone medication [20]. Some studies have suggested that eating soy foods can help reduce menopausal symptoms [21]. But others have suggested otherwise. The effects on midlife women are mixed [22]. Meanwhile the types of soy foods being consumed by Americans continued to expand and diversify. The way soy was being eaten impacted how beneficial it could be for your health. That means it natural, processed or fermented? [23]. Now as Americans are gaining continued to expand and diversify. The way soy was being eaten impacted how beneficial it could be for your health. That means it natural, processed or fermented? [23]. Now as Americans are gaining

Conclusion about EDC related health effects have to be made from epidemiological studies which can only reveal associations. By making inferences about human risk from experimental data from animal-or cell based models additional information is gathered. Traditional chemical testing of EDCs is inadequate to determine endocrine disrupting activity. They often test individual chemicals one at a time and assume individual chemicals have a “safe or acceptable” level of exposure. Tests are focused on adult animals, often mice and rats of different strains. They don’t reflect that endocrine disruptors can have an effect in very low concentrations in various tissues lifetime long. Concentrations in safety studies are mostly expressed in

The phthalates

Phthalates are a diverse and wide class of industrial chemicals. Since the 1940s they are used as plasticizers to make plastic more flexible and as stabilizing or solubilizing agents. They are non-natural EDCs.

Phthalates were first introduced in the 1920s when they replaced camphor as the favoured plasticizer for cellulose nitrate. The commercially availability of polyvinylchloride and the development of di (2-etylhexyl) phthalate started the boom of the PVC industry. Glycerol phthalate was the first synthetic polyester. It came already into use in World War I when it was used for waterproofing [29].

The phthalates or phthalate esters are esters of phthalic acid. Phthalates are made by reacting phthalic anhydride with alcohols that range from methanol and ethanol to tridecyl alcohol. They are divided in two distinct groups with very different toxicology, applications and classification based on the number of carbon atoms in their alcohol chains. Low molecular weight (LMW, 3-6 carbon atoms) are being gradually replaced for reasons of health concerns. They are replaced by high molecular weight (HMW) with more than 6 carbon atoms with increased permanency and durability. Since 2010 producers are increasingly forced to use non-phthalate plasticizers [29]. The phthalates also called the “everywhere chemicals” are released in the environment. Due to this ubiquity of plastics most Americans have metabolites of multiple phthalates in their urine. Finding a detectable amount of phthalates in the urine does not imply that the levels of one or more will cause an adverse health effect. The levels can be used for biomonitoring of populations. People are exposed to phthalates by eating and drinking foods that have been in contact with containers. Enteric coated pharmaceuticals are another source. To a lesser extent exposure can occur from breathing air that contains phthalates vapors or phthalate contaminated dust. Children have a greater risk because of their “hand to mouth” behavior and by playing with soft children toys. Epidemiological studies at the population level as in Denmark and other countries observing adverse trends in male reproducitivity together with declining sperm counts has led to the hypothesis of environmental contaminants being harmful to reproduction [30]. It was in the 2000s more epidemiological studies then questioned a link between the rising incidence in cryptorchidism, hypospadias, testes cancer and sexual precocity [31,32]. Although the presence of phthalate metabolites in urine does not imply an adverse health effect the above mentioned epidemiological studies, having the DES scandal in mind, resulted in extensive research on the effects of phthalates on the endocrine system [33,34].
parts per billions (PPBs) that can be substantially higher than levels of the natural hormones however [35]. Phthalate exposure is linked to genital abnormalities in boys, reduced sperm counts, decreased "male typical" play in boys, endometriosis and the metabolic syndrome including obesity. The androgen\textit{receptor} (AR) is believed to play a central role in this.

In 2009 a small Taiwane study in humans showed that phthalates passed from mother to fetus through the placenta, long be held as a natural barrier, affect female babies sometimes resulting in abnormal sexual development [36]. Pregnant women exposed to phthalates in the workplace were found to be two to three times more likely to deliver boys with the reproductive birth defect known as hypospadia [37]. Boys who are exposed to higher levels of certain types of phthalates in the womb may show less masculine behavior, measured by playing with trucks and play fighting, than boys who are exposed to lower levels [38]. A 2007 study found that higher levels of phthalates detected in the urine of adult males was associated with increased waist circumference and insulin resistance [39]. A 2009 study determined that phthalate exposure correlated with premature breast development in young Taiwanese girls [40]. The health risks of medical devices containing phthalates were reviewed for the first time in 2001 [41]. Several associations were identified with short term and long term health dangers mainly subfertility, bronchopulmonary dysplasia, , parenteral nutrition associated cholestatis, necrotising enterocritis and neurodevelopment disorders. These data were mainly based on anima lor human observational studies. In 2006 and 2007 the metabolism of the phthalates in humans was studied [42,43]. In 2010 and 2011 reports about the health risks of phthalate containing blood bags appeared [44-45]. The use of alternative plasticizers is recommended. In 2015 blood bags became "EHDP free" [46]. Pharmaceuticals that are enteric coated contain phthalates too. It took to 2017 before a Chinese study addressed this subject [47]. Definite associations couldn’t be found in this heterogenous study population. The phthalates, EHDP and dibutiryl phthalate have profound impacts on the male reproductive system similar to flutamide, an antiandrogen or androgen receptor antagonist. DEHP has a toxic effect by the metabolite monoethylhexyphthalate (MEHHP) on both Sertoli cells in the testes of the developing animal. Sertoli cell damage results in decreased sperm production which may be permanent even if the toxic agent is discontinued. The normal Sertoli cell development is under the influence of FSH (Folllicle Stimulating Hormone) The Sertoli cell has FSH receptors on its cell membrane. Probably these receptors are blocked by phthalates. Granulosa cells of the ovary responsible for producing estrogen are also dependent on FSH stimulation for normal function. Phthalates decrease estrogen production in female animals. In the testis the Leydig cells secrete testosterone and estradiol while the Sertoli cell, responsible for spermatogenesis, secretes Inhibin B. The phthalate DEHP does not bind to the androgen receptor (AR) but in animals in utero exposure to phthalates disrupts the differentiation program of the androgen dependent tissues [48]. Phthalates have been shown to reduce testicular testosterone levels in fetal and neonatal rats. The decreased testosterone production has been assiated with the downregulation of genes involved in steroidogenesis [48,49]. Concerns among the public and professional medical societies about the phthalates as endocrine disruptors are seriously. Recently four advocacy groups claimed that the phthalate concentrations in powder of Mac and cheese were more than four times higher than in block cheese and other natural cheeses in all 30 investigated samples. The article appeared however in the New York Times and was not peer reviewed therefore. Concentrations of phthalates were not revealed0 [50]. The Endocrine Society urged the EU parliament recently in a press release to be transparent around EDC criteria [51].

**Bisphenol A**

Bisphenol A (BPA) was first synthesized in 1891. Edward Charles Dodd identified the estrogenic properties when he was in search of an estrogen. Later he succeeded in discovering diethylstilbestrol (DES), which he called "the mother substance" [52]. BPA never found use as a drug. Its future was in plastics. Early in the 1960s the First epoxyresins were synthesized using BPA [53]. BPA’s safety profile was defined by its commercial use in plastics and accordingly by its toxic rather than hormone-like properties. Because BPA migrates from epoxyresins and polycarbonates used in food packaging and production the FDA (Food and Drug administration) considered the chemical an indirect food additive until now [54]. In 1993 endocrinologists at Stanford University determined that BPA was leacking from polycarbonate flasks in their laboratory, while searching for an endogenous estrogen in yeast [55]. Vom Saal et al. exposed pregnant mice to "physiologically" doses of BPA under the safety levels of 50 microgram/kg/day. He showed a higher than expected and a stimulating effect on the mammary glands. In male mice he reported increased prostate weight. These new low dose studies challenged the concept that BPA was only a weak estrogen [56,57]. The phenol group is essential for the estrogen mimicking effect of BPA because of the similarity of the phenol group of both BPA and estradiol. Typically phenol containing molecules similar to BPA are known to exert weak estrogenic activity [58]. BPA has been found to bind to both of the nuclear estrogen receptors, alpha (ER) and beta (ER). It is 1000-2000 fold less potent than estradiol. BPA can both mimic the action of estrogen and antagonize estrogen, indicating that it is a selective estrogen modulator (SERM) or partial agonist of the ER. At high concentrations BPA also binds and antagonizes the androgen receptor (AR). In addition to receptor binding the compound has been found to affect Leydig cell steroidogenesis in the testes including 17-alphahydroxylase, 17, 20 lyase and aromatase expression and interfering with LH receptor-ligand binding [59]. The major human exposure route is diet, including ingestion of contaminated food and water [60]. In the workplace inhalation and dermal exposure are the most probable routes [61]. Free BPA is found in high concentrations in thermal paper and carbonless copypaper. Popular uses of thermal papers include receipts, tickets and labels [62]. BPA has been linked or associated with numerous body functions.

**Sexual function**

A 2010 study of 427 male workers in regions where high levels of BPA exposure existed showed a relationship between high urinary BPA levels and worsening male sexual function. There were significant decreases in sexual desire, erectile function, ejaculation strenght and overall satisfaction [63]. Meeker et al. concluded in 2010 that urinary BPA may be associated with declined semen quality and increased DNA damage in patients recruited through an infertility clinic, but confirmatry studies were needed [64]. BPA can decline testosterone levels in mice and inhibit their sexual behavior [65]. Mendiola et al. found urinary BPA levels correlated negatively with seminal fluid volume [70].

**Fertility**

BPA has been shown to affect many endpoints of fertility [66]. Several studies examined individuals undergoing infertility...
treatments as in vitro fertilisation (IVF) and measured BPA in relation to various reproductive endpoints such as ovarian response, fertilisation success, embryo quality and implantation failure. In a cohort of women recruited from the Massachusetts General Hospital (MGH) Fertility Center, who were undergoing IVF treatment a higher urinary BPA was associated with a poorer ovarian response (fewer oocytes per cycle and decreased E2) In another population of women from the MGH Fertility Center Ehrlich et al. found that higher urinary BPA again significantly correlated with lower serum E2 and oocyte yield. In this study oocytes matured less and there were fewer fertilized oocytes [67,68]. In a Californian study higher urinary BPA also correlated with a lessened ovarian response after hyperstimulation [69].

**Sex hormones**

Many studies have found changes in endogenous sex hormone concentrations as well as in sex hormone binding globulin (SHBG). Workers exposed to BPA had higher total urinary BPA levels in the presence of lower FSH in contrast to a study by Meeker et al. who found higher FSH levels as well as lower Inhibin B levels [71]. Takeuchi et al. tested healthy women and men as well as women with the polycystic ovary syndrome (PCOS) for serum BPA and hormone concentrations. Total testosterone (T) and free testosterone (FT) were significantly higher in men and PCOS women with high BPA levels than in normal women. Men had also significantly higher FSH and dehydroepiandrosterenedione (DHEAS) and lower E2 levels than non-PCOS women, while the PCOS women had significantly higher E2, LH and androstenedione concentrations. Both PCOS women and men had higher BPA serum concentrations than non-PCOS women [71]. Takeuchi et al also studied sex hormone concentrations in non-obese and obese women with and without PCOS as well as in women with women with hyperprolactinemia and women with hypothalamic amenorrhea. In all women BPA was positively correlated with total T, FT, androstenedione and DHEAS [72-73]. Kandaraki et al also found a significant correlation between BPA and elevated androgen concentrations in women with and without PCOS [74]. The studies in PCOS are difficult to interpret as elevated androgens are a hallmark of PCOS and also associated with increased BPA levels. Thus there is no way to attribute an association solely to either factor as they are correlated with each other.

**Endometriosis**

Studies of BPA and endometriosis have yielded inconsistent results and were limited by the participant sampling framework, small sample size or use of serum BPA, which has very low and transient concentrations compared to urinary BPA concentrations. However a 2014 study by Upsen et al. suggests increased urinary BPA levels are associated with pelvic but not ovarian endometriosis [75]. Serum BPA was significantly lower in endometriosal cancer patients [75].

**Breast cancer**

Although studies showed a stimulating effect of BPA on the mammary gland in animals and high BPA showed higher mammary densities a definite relationship between BPA and breast cancer can not be determined [79,80]. As breast cancer most likely take years to develop may be longitudinal studies measuring BPA in utero should be done.

**Miscarriages**

A 2003 study by Sugiura et al. showed some evidence of a relationship between recurrent miscarriages and BPA exposure in women. The authors suggested the increased incidence of miscarriages from BPA exposure may be due to an increase in chromosomal abnormalities due to meiotic disruption. However the study sample size was small [81].

Recent epidemiological studies showed a more definite association between BPA exposure and miscarriage risk. Sample size was relatively large, urinary BPA was adjusted for creatinine and there was a low limit of BPA detection [84]. Though two studies reported an association between BPA exposure the conclusion is inconsistent [82,83].

**Premature deliveries**

Only a few studies were done They show little evidence of a relationship between BPA exposure and prematurity [85-87].

**Birth weight**

Studies looking for a possible relationship between birth weight and BPA exposure have shown divergent results probably due to great variations in study design. Positive as well as negative correlations were found. Recalling of birth weight by parents was also a problem [88-91].

**Male genital abnormalities**

Male genital abnormalities such as shorter anogenital distance (AGD) have been associated with exposure to antiandrogenic disruptors in humans [92-94]. Studies linking cryptorchidism to BPA exposure are less convincing [95,96]. Based on the current evidence there does not seem to be a link between BPA exposure and cryptorchidism.

**Liver function**

Many questions remain to be addressed about the causal link between acute and chronic EDC exposure and the development of non-alcoholic hepatic steatosis (NAFLD) in humans This is primarily because NAFLD is multifactorial parallelling the increase in the incidence of obesity and diabetes mellitus [97]. In two other studies weak associations between BPAs and disturbed liver function tests were found [98,99]. Overall the evidence is not convincing.

**Type 2 DM**

In the NHANES 2003-2006 cohort no significant associations between diabetes were found [99]. Some animal studies show glucose intolerance and insulin resistance [100]. Wang et al found a correlation between urinary BPA and obese and insulin resistance [101].

**Obesity**

Various studies showed BPAs to interfere with adipogenesis, but few human studies are available [102,103]. Tese data are conflicting. Takeuchi et al. found increased BPA levels were associated with an increased BMI [Body Mass Index] in non PCOS women. In an earlier study, however there was no association between BMI and serum BPA [72,73]. In a puberty study Wolff et al. found significantly lower urinary BPA levels in girls with a high BMI [104]. Studies of the NHAENES cohorts showed higher urinary BPA was strongly associated with higher BMI and waist circumference. When race ethnicity was examined the relationship between BPA exposure and obesity was only seen in Caucasians [105,106].
In the CHAMACOS study, examining mother-child pairs pre-and postnatal BPA exposure and its association with fat and BMI was studied. In girls they found that increased prenatal BPA exposure was associated with decreased body fat at 9 years old. However Total urinary BPA in boys at 9 years old was positively correlated with increased BMI, waist circumference, fat mass, overweight and obesity [107]. It is clear that a “might be” link between obesity and BPAs need more longitudinal prospective studies.

The thyroid

Brücker-Davis et al studied thyroid function in mothers exposed to BPA and healthy newborns. There was a non–significant correlation between maternal BPA and TSH in newborns merely indicating a trend to hypothyroidism [96]. In the CHAMACOS cohort a higher maternal urinary BPA concentration was significantly associated with lower maternal T4 and negatively associated with TSH in boys, but not in girls. Other studies resulted in even more confusing offspring results ranging from a trend to hyperthyroidism, no effect or a trend to hypothyroidism after maternal BPA exposure but all without clinical significance [108,109].

Cardiovascular disease

Much of the literature about BPAs and cardiovascular health stems from the NHANES data. Lang et al found that a higher urinary BPA concentration was more frequently associated with a diagnosis of cardiovascular disease [110-113]. Higher urinary BPA concentrations were associated with hypertension, stable angina pectoris, coronary artery disease peripheral artery disease etc., Olsen et al found weaker connections between BPA exposure and coronary artery disease [114]. Hard cardiovascular endpoints as cardiovascular mortality were not tested.

Childhood behavior neurodevelopment

Several studies have reported altered behavior in children exposed to BPA in utero or before puberty, indicating disruption of the brain during critical windows. In the HOMES study Braun et al. tested pregnant woman at 16, 24 weeks and at birthgiving for urinary BPA. When the offspring was 2 years their behavior was evaluated using the validated Behavioral Assessment System for Children. In girls but not boys there were significant associations between high maternal BPA and increased externalizing behavior (hyperactivity, aggression) these associations were only significant for prenatal BPA values. These studies indicate disruption of neurodevelopment caused by in utero BPA exposure, especially in girls that seem to have long lasting effects [115].

In contrast Yolton et al used the HOMES cohort too but didn’t find a correlation between maternal BPA and neurobehavioral abnormalities at the age of 5 weeks [116]. Poreire et al studied African American and Dominican woman in the US. Spot urine samples were collected at 34 weeks gestation and from children 3–4 years old. High maternal urinary BPA was significantly associated with higher scores (i.e. more problems) for boys (aggressive, emotional) with trends of poorer scores in withdrawal and sleep problems. In girls higher urinary BPA was associated with lower scores. In studies of resin-based dental composites were found to affect behavioral in pubertal children. BPA fillings were leaking BPA at the start and possibly long time [118-120]. On the whole these studies suggest BPA exposure is associated in some way with neurobehavioral problems in children.

Childhood asthma

Higher prenatal exposure was associated with increased odds of wheeze in the child at 6 months of age when BPA exposure was high in the First 16 weeks of pregnancy. At 3 years of age the association had gone [121-123]. This suggests a critical window.

Immune function

Immune function was also shown to be negatively associated with BPA exposure. Clayton et al. found that urinary BPA was significantly correlated with antibodies to cytomegalovirus (CMV). Increased CMV antibody titers indicate a depressed immune system and can be an early marker of immune dysfunction in humans [124].

Oxidative stress and inflammation

Tarantino et al. looked at endpoints of chronic inflammation in women with PCOS and without in relation BPA exposure. PCOS women with higher BPA concentrations had increased markers of chronic inflammation as higher C reactive protein (CRP) and interleukin-6 (IL-6) [125]. Hong et al. examined a large Korean population. There was a positive correlation between urinary BPA and oxidative stress as measured by the reactive oxygen species malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHJG) However this was not significant after adjustments [126,127]. In contrast Yi et al. found a positive correlation between conjugated urinary BPA but not (8-OHJG). The sample was very small. Lastly Yang et al studied men and pre- and psemenopausal women. Only in postmenopausal women urinary BPA was associated with higher MDA, 8-OHJG and CRP suggesting that postmenopausal women may be more sensitive to the effects of BMA than premenopausal women and men [128].

Epigenetics

A few studies have found that BPA can change the epigenome or gene expression. Some of these effects may be more immediate than the long term development of other diseases. Increased BPA levels in serum or urine were associated in general with decreased methylation in these studies [129-133].

PCBs

The first polychlorinated bisphenyl (PCB)-like chemical was found as a by-product of coal tar. A year later in 1881 German scientists synthesized the first PCB in a laboratory. In 1935 Monsanto took over commercial production of Swan Chemicals. The electric industry used PCBs as non-inflammable replacement for mineral oil to cool and insulate industrial transformers and capacitors. PCBs were also used as heat stabilizers in cables and electronics to enhance the heat and fire resistance of PVC.

In the 1930s the toxicity of PCBs and other chlorinated hydrocarbons were recognized because of a variety of industrial accidents. In 1966 PCBs were determined as environmental contaminants [134,135]. Despite active research to its likely carcinogenic effect and an efficient ban on their production in the 1970s PCBs still persist in the environment because they are not biodegradable. PCBs belong to the group of organic pollutants (POPs). Other members of this group are DDT and the dioxines. People are still being exposed to PCBs from various sources such as caulk, some oil based paints and Floor finish used between 1950 and 1979 in schools. Eating PCB contaminated fish is the most common route of exposure to PCBs [138,139]. PCBs are called “possible
carcinogenic"by the EPA. Studies of PCBs in humans have found increased rates of melanoma, liver cancer, gallbladder cancer, biliary tract cancer, gastric, intestinal cancer and brain cancer. PCBs are known to cause a variety of cancer types in rats, mice and other study animals [136,137]. PCBs also cause neurodevelopmental abnormalities after exposure in utero with detrimental effects on neuropsychology [146].

The endocrine effects of PCBs are largely due to their effect on the thyroid. Many of the hydroxylated PCBs which are also biologically active have a high degree of structural resemblance to thyroxine (T4). Animal studies showed conflicting results regarding the influence of PCBs on the thyroid metabolism [146,147].

Zoeller et al provides the strongest "evidence to date" that PCBs present in flame retardants, clothes, paint, adhesives and electric transformers can interfere with the thyroid hormone action in pregnant women and may travel the placenta to affect the fetus. PCBs interfere with the way thyroid hormone function but don’t actually change the amount of hormone in the body. Although these effects are largely invisible in scientific studies that only measured thyroid hormone levels they may be having a real impact on the infant’s brain development. In this prospective birth cohort study he and colleagues looked at the effects of low-dose chemical exposure in 164 women. Tissue from their placenta's was analyzed for specific enzymes (CYP1A1) which change endocrine disruptors into a form that can interfere directly with the thyroid hormone receptor. It suggests that endocrine receptors should be studied more on the cellular level [146, 141]. CYP1A1 levels are higher in cigarette smokers. PCBs increase adipocyte differentiation in vitro and might increase body weight. They increase hepatic steatosis and visceral adiposity in the context of a lipid enriched diet [142,143] they belong to the "obesogens" that can increase hepatic steatosis and visceral adiposity in the context of a lipid enriched diet [142,143]. Turtles are sex reversed by PCBs. They showed estrogenic activity in this study [145]. There are over 259 PCBs, some with dioxin-like properties depending on their congeners. They can have estrogen agonist as well as estrogen antagonist effects [146-148]. Studies on the risk of endometrial cancer and PCBs have been rather elusive despite decades of research. Their carcinogenic effect would be due to the fact that they can induce potent epithelial cell activation, enhance cellular oxidative stress, stimulate increased permeability across the vascular endothelium and induction of adhesion molecules [149,150]. Epidemiological studies in humans don’t support an increased incidence of endometrial cancer despite the weak estrogen activity of PCBs [151].

In epidemiological studies the risk of breast cancer for PCBs exposure can’t be demonstrated. An Inuit study showed no change in the risk for breast cancer and POPs including PCBs in the absence of other risk factors for breast cancer [152-154].

In the male negative associations between PCBs and sperm count and circulating testosterone levels have been found [153]. A proven association between PCBs and testicular germ cell cancer have not been found [155]. For prostate carcinoma no increased risk for PCB scan be established. Neither due to the multifatorial origin of these tumors [156].

PCBs are believed to influence the immune system negatively [157,158]. In Dutch school children this resulted in a higher incidence of middle ear infections [159]. Children exposed to PCBs have also a reduced antibody response to vaccinations [160].

PCBs are thought to influence neurobehavior. The relationship between PCBs and neurodevelopment in later childhood are strongest for motor, cognitive, usual recognition and executive functions including ADHD and ASD-like behaviors [162]. There is an inverse correlation between PCBs exposure and IQ [161].

Dioxin and dioxin-like compounds

Dioxin and dioxin-like compounds are environmental persistent organic pollutants (POPs). The POPs consist of the polychlorinated dibenzo- p dioxins or simply dioxins, the polychlorinated dibenzo-p-dioxins and the polychlorinated polybrominated phenyls. The history of dioxin production and dioxin poisoning is nearly 200 years old and dates back to a production plant in the state Hessen, Germany [163]. Chloracne, a persistent cystic and hyperkeratotic skin condition was first identified in German industrial workers and remains a hallmark of dioxin exposure [164]. The use of PCBs began in the early 20th century with commercial production inevitably also of mixtures that contain dioxin-like PCBs. The history of diox knows many accidents and disasters.

In 1947 X-disease a hyperkeratotic skin condition akin to chloracne was first reported in American cattle. A decade off X-disease a massive die off of commercially raised chickens in the US occurred due to a condition referred as "chicken edema" later found to be caused by TCDD contamination of chicken food supplies [166]. Between these two animal poisonings an explosion in a Monsanto plant resulted in the exposure of workers in the dioxin-contaminated herbicide 2, 4, 5, T and persistent chloracne was observed [167]. Later studies on these and other occupational dioxin exposed workers demonstrated an increase in all cancers combined in the most highly exposed workers [168]. Between 1962 and 1970 "Agent Orange" containing the same herbicide contaminated with TCDD was used as a defoliant in the Vietnam War. The use of "Agent Orange" was associated with an increased risk of diabetes and multiple cancers with increased duration of exposure [169]. The Institute of Medicine found sufficient evidence for a connection between "Agent Orange" exposure and soft tissue sarcoma, nonHodgkin lymphoma, Hodgkin disease and chronic lymphocytic leukaemia [170]. In 1968, in Kyushu, Japan a rice bran oil company supplies became contaminated with PCBs and PCDFs. And contaminated with dioxins. The contaminated oil resulted in the death of hundreds of thousands of birds. In humans skin lesions, fatigue and altered reproductive and immunologic function were symptoms of what was referred to as “Yusho” (oil) disease and development delays were observed in children [171]. Unfortunately this incident was repeated in Taiwan and was referred to "Yucheng disease" (oil). In this event the association between gestational or lactational exposure was associated with impaired cognitive development and behavioral problems and altered reproductive parameters post puberty among the exposed males [172]. In the late 1970s the Great Lakes region noticed greatly diminished reproduction among lake trout and mink which has persisted until now [173]. Humans living in the area have shown signs of both developmental and immunologic consequences of these persistent organic pollutant via dietary fish intake and breast milk [174]. In 1976 an explosion occurred at an Italian chemical plant in Seveso producing 2, 4, 5-T. Skin lesions as chloracne occurred in several weeks. In the years that followed continuing studies of the population showed TCDD acts as a carcinogen in humans and increased the risk for diabetes, adverse cardiovascular effects and altered endocrine function [175,176]. U. S’ EPA issued its First health assessment of TCDD in 1985 and 1991 [177]. After these reports the last events were a contaminated live stock food supply...
and a contaminated chocolate withdrawal in Belgium. The murder at the Ukraine politician Victor Yushchenko was the last incident that draw massive public attention [178-180]. Dioxins and dioxin-like compounds exert their biochemical effect through activation of the Aryl hydrocarbon receptor [AhR], a ligand activated transcription factor and member of the PAS superfamily of transcription factors [186]. In the absence of dioxin signalling the AhR may be involved in cell cycle control and tumor suppression in the stomach, prostate and hematopoietic tissues [181,182].

Adverse effects of dioxins on human health may include

- Cardiovascular disease, diabetes, cancer, porphyria, endometriosis, early menopause, reduced thyroid hormones and testosterone, altered immunological response, skin, tooth and nail abnormalities, altered growth factor and altered metabolism [183]. A study in men 30 years after the Seveso disaster found that men with pre-puberty exposure had reduced sperm count and motility while those with post-exposure puberty had increased sperm counts and motility suggesting a critical window [184-188].

- Thyroid stimulating hormone (TSH) levels were elevated in neonates born from mothers with persistently elevated plasma dioxin levels nearly 30 years after exposure, indicating hypothyroidism [185]. This illustrates again the significance of the timing of dioxin exposure with respect to the outcomes [189,190].

**DDT--pesticides**

DDT was the best known of several chlorine containing pesticides used in the 1940s and 1950s. DDT was first synthesized in 1874 by Othmar Zeidler and Adolf von Bayer [191]. DDT's insecticidal properties were not discovered until 1939 when the Swiss chemist Paul Hermann Müller reported this. For this discovery he was awarded the Nobel Prize in Physiology and Medicine in 1948. DDT was used during World War 2 to control the insect vectors of typhus, nearly eliminating the disease in Europe and the South Pacific. It was sprayed aerally for malaria and dengue control with spectacular effects [192,193]. In 1945 DDT was made available to farmers as an agriculture insecticide [192-194]. A WHO program directed at eliminating malaria with DDT only succeeded in areas with high socio-economic status and well organized healthcare systems [195]. For this reason DDT was not used in sub-Saharan Africa which still has the greatest malaria burden now [195]. DDT was less effective in tropical regions due to the continuous life cycle of mosquitoes and the poor infrastructure. Resistance to drug treatment evolved rapidly. Eradication programs were abandoned in 1969. Spraying programs using DDT were curtailed due to concerns over safety and environmental effects [195,196]. DDT is a persistent organic pollutant (POP) that is readily absorbed in soils and sediments which can both acts as sinks or as long term exposure affecting organisms. Depending on conditions its soil half life ranges from 22 days to 30 years [197]. Because of its lipophilic properties DDT can bioaccumulate especially in predatory birds [198]. The chemicals and its breakdown products DDD and DDE caused egg shell thinning and populations of predaceous preys, including the Bald Eagle, rapidly declined in North America and Europe [199]. The mechanism of egg shell thinning is not known [200]. DDT is an endocrine disruptor that acts as a weak androgen receptor antagonist but not as an estrogen [201]. In the 1970s and 1980s agriculture use of DDT was banned in most developed countries. In 2004 the Stockholm Convention put a global ban on DDT and several other POPs [202]. Despite the world wide ban agriculture use of DDT continues in India, North Korea and possibly elsewhere [203-205].

The organochlorine pesticides have been associated with endocrine disorders Multiple observations in animals in polluted areas demonstrated a significant increase in goiter and thyroid imbalance. In humans exposed to background levels of chemicals DDT had no significant effect on thyroid function [204]. There is no clear association between breast cancer and DDT or DDE [206]. DDT is rated as “possible carcinogenic” by the International Agency for Research on Cancer (IARC:199). This rating was largely based on the induction of liver tumors in experimental animal studies. Most human studies reviewed by the IARC in 1991 did not show an association between DDT and cancer risk. Research has not supported an association between DDT or DDE and the incidence of colorectal, lung, bladder, prostate, endometrial and stomach cancer [207].

The prevalence of diabetes is positively correlated with plasma DDE levels. However given the high correlations with other organochlorine exposures additional research is needed to delineate the specific contributions of DDT and DDE [208]. High DDE levels are associated with an increased rate of fetal loss [209]. Most studies found no association between maternal serum DDE levels and fetal growth, gestational duration, premature labor, birth weight or head circumference [210]. Associations between DDE levels and early weaning of lactation are spurious and further research is warranted [211]. The effects on urogenital birth defects are fairly consistent with a two times higher risk for cryptorchidism and hypospadia [212]. Male fertility studies showed that plasma DDE concentrations were negatively correlated with sperm motility and decreased sperm count [213]. Female fertility studies show that DDT exposure may delay time to pregnancy [214]. Neurodevelopmental have shown that high DDT exposure was associated with poorer psychomotor development, poorer cognitive memory and poorer quantitative verbal and executive functions [215]. DDT and particularly DDE have demonstrated the potential for modulating the human immune response as measured by multiple markers such as interleukin 4, 13 and plasma levels of interferon-gamma, white blood cell counts, various lymphocytic phenotypes and immunoglobulin (Ig) A, G and E levels [216]. Associations with aplastic anemia, otitis media, farmers lung and asthma were reported [217]. POPs including DDT may be released from fat tissues during weight loss crash diets [218].

**Glycophosphates**

Glyphosate is a broad spectrum herbicide and crop dissecant. It is an organophosphorus compound madeto kill weeds, especially broad leaves, weeds and grasses that compete with crops. It was first synthesized in 1950 by the Swiss chemist Henry Martin, who worked at the Swiss company Cilag. The work was never published. In 1970 glyphosate was reinvented by the Monsanto chemist John E. Franz. Monsanto brought it to the market in 1974 [219]. It is also a chelator binding calcium, magnesium, manganese, copper and zinc. Glyphosate kills plants by interfering with the synthesis of the aromatic amino acids Phenylalanine, tyrosine and tryptophan in the shikimate pathway. By inhibiting this pathway energy and resources of the plant are diverted away. Growth stops within hours and it takes several days for the leaves to begin turning yellow [221]. The half life of glyphosate in soil ranges between 2 and 197 days. The median half life in water varies from a few days to 91 days [222]. Glyphosate formulations contain a number of adjuvants which are trade secrets [223].

Despite a review article in 2014 that reported a two fold increased

risk for glyphosate exposure and B cell lymphoma the discussion about its possible carcinogenic potential is still going on widely [224]. Several studies have not found mutagenic effects of glyphosate while others did. Because the EPA lists glyphosate as non-mutagenic it is also not listed in the EPA International Research on Cancer Data Bases [225]. The IARC (International Agency for Research on Cancer) finalized a meeting at March, 2015 with the statement that glyphosate is “probably carcinogenic” to humans. The EPA is supposed by law to review the data once in a 15 years time and should do this again in 2017. The WHO has declared glyphosate as “possible carcinogenic” and the State of California recently put glyphosate under Proposition 65 as “Known to the State to Cause Cancer” [226]. In 2007 the EPA selected glyphosate for further screening through its Endocrine Disruptor Screening Program (EDSP) and concluded there was no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathway [227]. However some researchers found a weak estrogenic activity at the estrogen receptor depending on the assays used [228].

**Neonicotinoids**

The neonicotinoids are known since 1970. The precursor to nithiazine was first synthesized by Henry Feuer, a chemist at Purdue University [229]. In 1984 niathiazines mode of action was settled as a postsynaptic acetylcholsterol receptor agonist. [230]. Nithiazine is not photostable and became therefore not commercially available. In 1985 Bayer patented imidaclopid as the first commercial Endocrine Disruptor Screening Program (EDSP) and concluded there was no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathway [227]. However some researchers found a weak estrogenic activity at the estrogen receptor depending on the assays used [228].

**Heavy metals as EDC**

Potential candidates are cadmium(Cd), mercury(Hg), arsenic [As], lead [Pb] manganese[Mn] and Zinc[Zn]. The mechanisms of action are largely to be elucidated Cadmium and arsenic have been studied mostly. Cadmium could be a metalloestrogen but this has not been studied well. Animal studies use frequently toxic levels and the co-occurrence with other EDCs is not studied well. Further studies to delineate the role of heavy metals are needed therefore [228].

**Conclusion**

Endocrine disruptors are for a long time among us, some for more than 100 years. Research in this area has been performed only for a few decades Central in this research is the time of exposure, in utero and early childhood which is of critical importance. The critical window is often 20 years or more The research trusts on associations and correlations which suggest a plausible cause. For a proven more definite causal relationship EDCs should be eliminated or replaced by law. Observing a declining incidence of of testes cancer or an improvement of sperm quality in the next 20-30 years would make the circle round [239].

**References**


to vaccinations in children exposed to polychlorinated biphenyls. PLOS Medicine.


