NUCLEAR RECEPTORS & ENDOCRINE / METABOLIC DISRUPTION

Jack Vanden Heuvel, INDIGO Biosciences Inc., State College, PA

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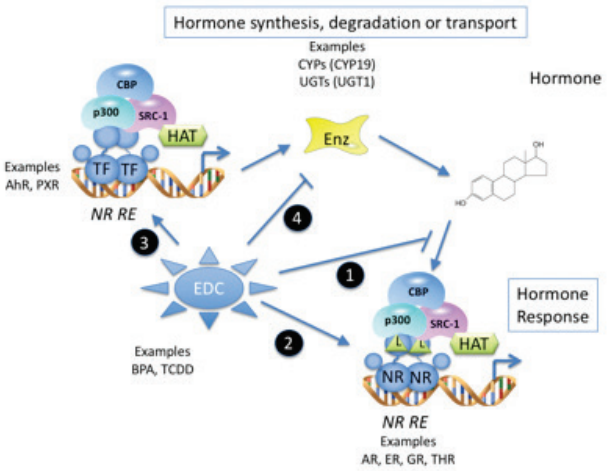
1. Overview

There are many important environmental chemicals that are of concern due to their ability to affect the endocrine system, so-called endocrine disrupting chemicals (EDCs). Compounds that cause endocrine disruption may do so by altering drug and xenobiotic metabolic processes; hence, a subgroup of EDCs may also be considered "metabolic disruptors" as well. In this review, we will discuss some important EDCs and their mechanism of action, in particular focusing on their interaction of nuclear receptors (NRs). Much of this information comes from recent review articles on the subject (1-6). Endocrine disruptors are exogenous compounds with the potential to disturb hormonal regulation and the normal endocrine system, consequently affecting health and reproduction in animals and humans.

Figure 1 Mechsnism of endocrine disruption



Endocrine disruptors can interfere with the production, release, metabolism, and elimination of or can affect the activity of natural hormones (see Figure 1).

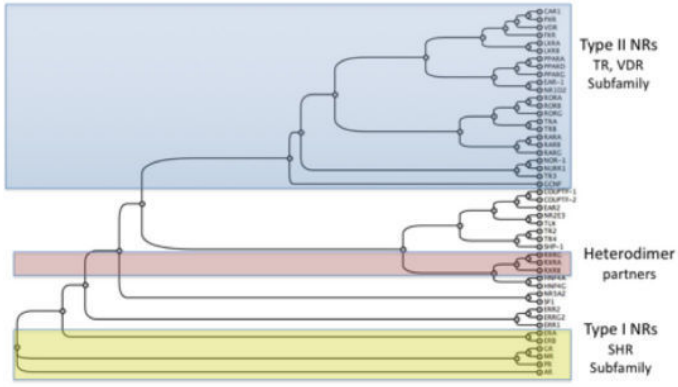
a. Endocrine Disruption

There are four main ways that EDCs can affect endocrine or metabolic processes: 1). Inhibit binding of the natural hormone to the ligand binding domain of a NR (competition for binding); 2). Affect NR signaling via partial agonist response, coactivator activity, DNA structure or competitive transcription factor association; 3). Affect the expression of enzymes involved in hormone synthesis, degradation or transport; and, 4). Directly effect on activity of enzymes involved in hormone synthesis, degradation or transport.

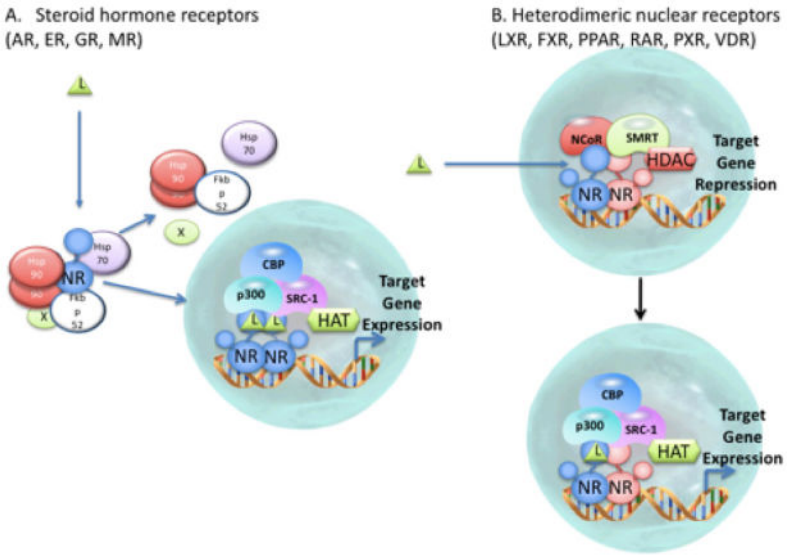
Endocrine disruptors may also be derived from natural animal, human, or plant (phytoestrogen) sources; however, for the most part international concern is currently focused on synthetic chemicals and EDCs. This concern is further amplified by two factors, the expansion in chemical production, which has now reached 400 million tons globally, and the increased pollution from these chemicals. As such, the impact on human health through known or unknown effects of these chemicals on hormonal systems is great.

b. Metabolic Disruption

This could be considered a subdivision of Endocrine Disruption. In addition to the developmental and reproductive effects, there is also a growing concern that metabolic disorders may be linked with EDCs. As has been widely reported, global obesity rates have risen dramatically over the past three decades in adults, children, and adolescents, especially in developed countries. Obesity is frequently associated with metabolic disorders (including type 2 diabetes, metabolic syndrome, cardiovascular and pulmonary complications, and liver disease) as well as other health issues, including reproductive defects, and some forms of cancer.



A combination of genetic, lifestyle, and environmental factors likely account for the rapid and significant increase in obesity rates. However, the increased incidence of metabolic diseases also correlates with substantial changes in the chemical environment resulting from new industrial and agricultural procedures initiated over the past 40 years. This change in the environment has led to the hypothesis that some of the numerous environmental pollutants are EDCs, interfering with various aspects of metabolism and adding another risk factor for obesity. This hypothesis is supported by laboratory and animal research as well as epidemiological studies that have shown that a variety of environmental EDCs can influence adipogenesis (fat cell differentiation) and obesity. Such EDCs have been referred to as **environmental obesogens**. However, because adverse effects by EDCs may also lead to other metabolic diseases such as metabolic syndrome and type 2 diabetes, this subclass of EDCs would be better referred to as **metabolic disruptors**.



2. A Common Molecular Mechanism for Endocrine Disruption and Metabolic Disruption

Hormones function mainly through interactions with their cognate receptors, which can be classified into two large groups: (a) membrane bound receptors, which respond primarily to peptide hormones such as insulin, and (b) nuclear receptors (NRs), which are activated by interaction with small lipophilic hormones such as sex steroid hormones. EDCs may possess multiple mechanisms of action; however, because many EDCs are small lipophilic compounds, one privileged route is through their direct interaction with a given NR, which presumably perturbs or modulates downstream gene expression. For example, most EDC associated reproductive and developmental defects are thought to result from EDCs interfering with the function of the estrogen receptor (ER) and/or androgen receptor (AR), thereby disrupting the normal activity of estrogens and androgens ligands.

In humans, the NR superfamily encompasses 48 members that share a common structure and, once activated, bind as dimers to specific response elements located near target gene promoters (see Figure 2). These dimers may be homodimers or heterodimers with retinoid X receptor (RXR), another member of the NR superfamily. In addition to the sex steroid receptors, the NR superfamily includes transcription factors that play pivotal roles in the integration of the complexities of metabolic homeostasis and development. The ability of EDCs to interact with these NRs is supported by, and explains, the wide range of metabolic perturbations reported in both experimental and epidemiological studies. It also reinforces the concept of associating endocrine and metabolic disruption.

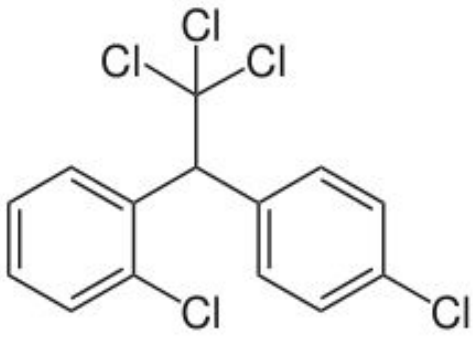
The first section discusses the chemical compounds that are presently considered to be major potential endocrine/metabolic disruptors. Also summarized is the impact of these chemicals on human health and metabolism on the basis of available epidemiological studies. The last section will examine a case-study.

3. Example endocrine and metabolic disruptors

EDCs encompass a variety of chemical classes, including pesticides, compounds used in the plastic industry and in consumer products, and other industrial by-products and pollutants. They are often widely dispersed in the environment and, if persistent, can be transported long distances; EDCs are found in virtually all regions of the world. Persistent organic pollutants (POPs) are prevalent among environmental contaminants because they are resistant to common modes of chemical, biological, or photolytic degradation. Moreover, many EDCs can be stored for years in animal and human fat mass. However, other EDCs that are rapidly degraded in the environment or the human body, or that may be present for only short periods of time, can also have serious deleterious effects if exposure occurs during critical developmental periods.

EDCs can be categorized according to their intended use (e.g., pesticides) or their structural properties (e.g., dioxins). The main categories of chemicals with suspected metabolism disrupting activity are presented below (the majority of this information is from reference 1).

a. Pesticides



Pesticides are any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. Several hundreds, if not thousands, of different chemicals are used as pesticides, and human exposure to these pesticides is widespread. Prominent chemical families include organochlorine pesticides (OCPs), organophosphates, carbamates, triazines, and pyrethroids. All OCPs are persistent. Even though OCPs such as the insecticide dichlorodiphenyltrichloroethane (DDT) are currently banned in most developed countries and were subsequently replaced in 1975 by organophosphates and carbamates, DDT contamination still exists. OCPs are detected in human breast milk and adipose tissue and may **exhibit estrogenic, antiestrogenic, or antiandrogenic activity**. A well-documented case study in the United Kingdom listed 127 pesticides identified as having endocrine-disrupting properties. With respect to metabolic disorders, a large number of epidemiological studies have also linked pesticide exposure with obesity, diabetes, insulin resistance, and metabolic syndrome. For example, an association was discovered between prenatal exposure to the DDT breakdown product dichlorodiphenyldichloroethylene (DDE) and increased body mass index in adult women. Similarly, cord blood levels of the OCP hexachlorobenzene correlated with a two- to threefold-higher risk of an elevated body mass index and obesity in children. Another study used the National Health and Nutrition Examination Survey (NHANES) database and carried out a cross-sectional analysis of 1,721 adults; the study reported a positive association between diabetes and the levels of 19 different persistent pollutants (including OCPs) measured in serum. Other epidemiological studies find a significant association between pesticide exposure [mostly OCPs such as heptachlor epoxide, oxychlordane, or β- hexachlorocyclohexane (β-HCH)] and higher incidences of metabolic syndrome, insulin resistance, and diabetes. A higher prevalence of diabetes is also associated with DDE exposure.

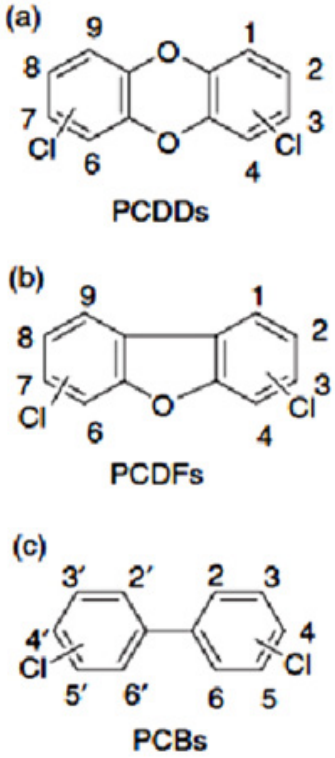
Table 1 Organochlorines (e.g., DDT)

Type or Source	Pesticides and plasticisers
Legal Status	1970s, DDT banned in most developed countries; 2000s, restricted under the Stockholm Convention
NRs (Mode of action)	Estrogen Receptor α (ERα), Androgen Receptor (AR)
In vitro/Animal Studies	Carcinogen (leukemia), reproductive effects
Human Epidemiology Studies	Associated with Metabolic Syndrome (MetS) and diabetes
Developmental Exposure Studies	Associated with children being overweight
Human Exposure	Banned. Soil-half life 22 to 30 yrs
Levels in human body	DDE, variable, range <5 to 15,000 mg/kg
Biological half-life	5 years
Concentration experimentally used	In cells 20 μM DDT

Adapted from: Casals-Casas, C., and Desvergne, B. (2011) Endocrine disruptors: from endocrine to metabolic disruption, Annu Rev Physiol 73, 135-162.

b. "Dioxins"

"Dioxins" consist of a group of organochlorines that include the polychlorinated dibenzodioxins (PCDDs), the polychlorinated dibenzofurans (PCDFs), and the polychlorinated biphenyls (PCBs) and other related compounds. Polycyclic aromatic hydrocarbons (PAHs) with dioxin-like activity can be produced from natural sources such as volcanoes and forest fires but are created mostly by human activity as by-products in organochloride production, in incineration of chlorine-containing substances such as polyvinyl chloride (PVC), and in bleached paper production. The PCDD 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic of all dioxins. This dioxin was a major contaminant in the Seveso catastrophe and in the Vietnam War (Agent Orange); it was also used as a poison in the attempted assassination of Viktor Yushchenko. Dioxins are fat soluble and readily climb the food chain via their bioaccumulation in fat tissues. They are neither readily metabolized nor excreted, and TCDD has a half-life of approximately 8 years in humans.



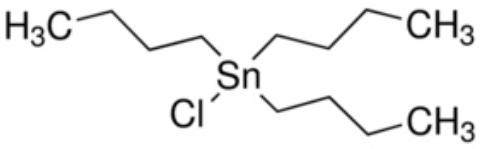
Several epidemiological studies have evaluated the toxic effects of TCDD and others dioxins on the general population as well as heavily exposed subgroups such as Vietnam War veterans. These studies demonstrate a cause and effect between dioxin exposure, with an increase in cancers, nervous system degeneration, immune damage, thyroid disease, and reproductive and sexual development disorders. With respect to metabolism, exploration of the NHANES database indicated that PCDDs and PCDFs are weakly associated with metabolic disorders, whereas PCBs are strongly associated with type 2 diabetes.

Table 2 Dioxins (e.g., TCDD)

Type or Source	Environmental pollutants in food
Legal Status	1970s, PCBs banned and others restricted under the Stockholm Convention
NRs (Mode of action)	Mainly arylhydrocarbon receptor (AhR); indirect with peroxisome proliferator-activated receptor ⍰ (PPAR⍰), ER⍰
In vitro/Animal Studies	Cancer (liver, skin); reproduction, Adipogenesis inhibition
Human Epidemiology Studies	Chlorance, cancer, associated with MetS, obesity and diabetes
Developmental Exposure Studies	
Human Exposure	TDI: 1–4 pg/kg
Levels in human body	In adipose tissue: 3.6 pg/g lipid In blood: 2.2 ppt
Biological half-life	7-11 years
Concentration experimentally used	In mice: doses of 5–500 ng/kg/day

Adapted from: Casals-Casas, C., and Desvergne, B. (2011) Endocrine disruptors: from endocrine to metabolic disruption, Annu Rev Physiol 73, 135-162.

c. Organotins



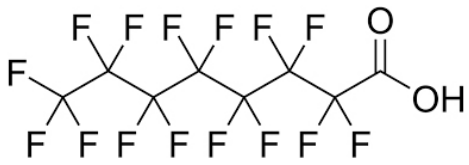
Organotins, including tributyltin chloride (TBT) and bis(triphenyltin) oxide (TPTO), are persistent organic pollutants that have been widely used as agricultural fungicides, as rodent repellents, as molluscicides, and in antifouling paints for ships and fishing nets. Organotin compounds such as PVC are also used to stabilize plastics. TBT and TPTO provide one of the clearest examples of environmental endocrine disruption: Exposure of marine gastropods to very low concentrations of these compounds induces an irreversible sexual abnormality in females termed imposex, resulting in impaired reproductive fitness and possibly sterility. Concerns over the toxicity of these compounds led to a worldwide restriction and a ban on marine uses. Currently, human exposure may come from dietary sources, such as fish and shellfish, or through contaminated drinking water and food. However, no epidemiological data are available concerning human exposure, although TBT has been reported to have modest adverse effects on mammalian male and female reproductive tracts. Nonetheless, recent experimental studies revealed proadipogenic activity of TBT and TPTO.

Table 3 Organotins (e.g., TBT)

Type or Source	Environmental pollutants in food
Legal Status	Banned worldwide
NRs (Mode of action)	Retinoid X receptor (RXR); PPAR
In vitro/Animal Studies	Adipogenesis induction, reproductive effects
Human Epidemiology Studies	
Developmental Exposure Studies	Control of adipogenesis disruption (mice)
Human Exposure	TDI: 1.6 mg/kg
Levels in human body	In serum: 27 nM In human tissue: 3–100 nM
Biological half-life	From 23 to 30 days
Concentration experimentally used	In mice: induce adipogenesis at 0.05–0.5 mg/kg

Adapted from: Casals-Casas, C., and Desvergne, B. (2011) Endocrine disruptors: from endocrine to metabolic disruption, Annu Rev Physiol 73, 135-162.

d. Polyfluoroalkyl compounds



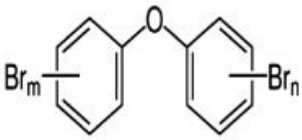
Polyfluoroalkyl compounds (PFCs) are synthetic fluorinated organic compounds used in a wide range of industrial applications and consumer products, including paper, leather, textile coatings, and fire-fighting foam, and in the polymer industry. Among them, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are widely detected in the environment. PFCs are classified as persistent organic pollutants, even though they are not stored in fat tissue but instead form chemical adducts with liver and serum proteins. Laboratory rodents exposed to PFCs exhibit developmental effects such as reduced birth weight and increased neonatal mortality. In addition to hormonal perturbations with decreased testosterone levels and increased estradiol levels in adult rats, reductions in serum cholesterol and/or triglyceride in mice and rats exposed to high doses of PFOS and PFOA suggest that these chemicals disturb normal lipid metabolism. Inverse relationships were observed between PFOS and PFOA concentrations in cord blood and birth weight, ponderal index, and head circumference in children. Numerous studies have evaluated the possible associations of blood PFC levels with metabolic parameters. Weak association with increased cholesterol (HDL). Most of these studies are cross-sectional and as such fail to provide a causal link but rather draw attention to the potential effects of PFCs on human physiology.

Table 4 Polyfluoroalkyl compounds (PFCs) (i.e. PFOA, PFOS)

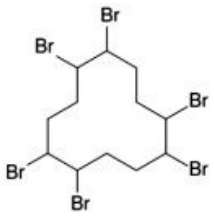
Type or Source	Plasticizer, food contaminant, fabric coating
Legal Status	Restricted worldwide
NRs (Mode of action)	PPARs, TR, CAR
In vitro/Animal Studies	Cancer (liver, testis, pancreas), hypolipidemia, weight loss, anorexigenic effect
Human Epidemiology Studies	Associated with increased cholesterol levels
Developmental Exposure Studies	Weight gain and increased serum insulin and leptin levels (mice)
Human Exposure	Indoor air levels: PFOS: 5 ppm . PFOA: 3.7 ppm
Levels in human body	Serum-level medians: PFOS: 19.9 µg/L PFOA: 3.9 µg/L
Biological half-life	PFOS: 5.4 years PFOA: 3.8 years
Concentration experimentally used	In rodents: PFOA prenatal exposure effects in a range of 0.01–5 mg/kg

Adapted from: Casals-Casas, C., and Desvergne, B. (2011) Endocrine disruptors: from endocrine to metabolic disruption, Annu Rev Physiol 73, 135-162.

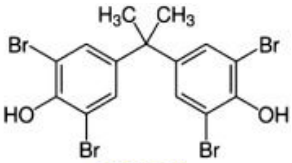
e. Brominated flame retardants



PBDE



HBCD



TBBPA-A

Brominated flame retardants (BFRs), particularly polybrominated diphenyl ethers (PBDEs), are additives used as flame retardants in a great number of consumer products such as house electronic equipment, clothing, and furniture. Although these compounds have decreased fire incidences, they are highly prevalent, ubiquitous, and persistent pollutants. The main sources of human exposure come from indoor environments, diet, and occupational exposure. Despite their beneficial effects, these chemicals are also thought to adversely affect human health through endocrine disruption and developmental neurotoxicity. In addition, increased incidences of hepatocellular carcinoma and thyroid adenoma have been observed in rodents, albeit with relatively high exposure doses. Taken together, these observations have led to the recent ban of PBDEs in several American states and in the European Union, where a ban on all BFR use is being contemplated. Meanwhile, PBDEs are still present in environmental samples and are detected in milk, serum, and adipose tissue in animals and humans.

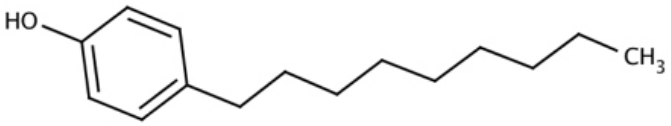
Published studies addressing the consequences of human BRF exposure remain scarce, and the effects of BFR exposure on sex steroid hormone systems are still poorly understood. One study demonstrated a correlation between PBDEs in breast milk and congenital cryptorchidism, although confounding factors may have been present. Only a few epidemiological studies have addressed the possible impacts of these persistent pollutants on metabolic parameters in humans. The most telling study revealed that, among six different BFRs, PBDE-153 and the polybrominated biphenyl PBB-153 showed an inverted U-shaped association with type 2 diabetes and metabolic syndrome. Clearly, more studies are needed to clarify the possible extent of BFR-related damage.

Table 5 Brominated flame retardants (BFRs) (i.e. PBDE)

Type or Source	Flame retardant
Legal Status	PBDE banned in the EU and some U.S. states
NRs (Mode of action)	Pregnane X receptor (PXR), ERs, thyroid hormone receptor (TR)
In vitro/Animal Studies	Cancer (liver, thyroid), Lipolysis increase, glucose oxidation decrease
Human Epidemiology Studies	Associated with MetS and diabetes
Developmental Exposure Studies	
Human Exposure	Diet: 37–97 ng/day; air through dust, Adults: 16.7 ng/day; children: 191.3 ng/day
Levels in human body	Mean levels in adipose tissue: Europe and Asia: <5 ng/g lipid North America: >200 ng/lipid In fetal liver: range of 4–98.5 ng/g liver; In breast milk: range of 1.57–73.9 ng/g liver America: >200 ng/lipid
Biological half-life	In serum: from weeks to months
Concentration experimentally used	In rats: exposure to 14 mg/kg/day for four weeks alters lipolysis and glucose oxidation

Adapted from: Casals-Casas, C., and Desvergne, B. (2011) Endocrine disruptors: from endocrine to metabolic disruption, Annu Rev Physiol 73, 135-162.

f. Alkylphenols



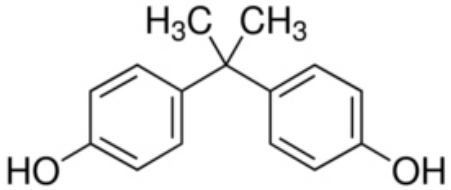
Alkylphenols such as 4-n-nonylphenol and 4-n-octylphenol are surfactants widely used in detergents, emulsifiers, antistatic agents, demulsifiers, and solubilizers and are found commonly in wastewater. They are also used as additives to plastics such as PVC and polystyrene, from which they can leach. Alkylphenols are capable of initiating proliferation in breast tumor cells in the laboratory, consistent with their capacities for estrogenic and antiandrogenic activity. However, given the low environmental concentrations and the current regulation of alkylphenol use, some argue that alkylphenols do not pose a health risk. Human exposure was recently evaluated in a population of women in southern Spain; nonylphenol was detected in 100% of the adipose tissue samples tested. In this study, body mass index was associated with nonylphenol levels and emerged as a determinant of exposure. More studies with larger study populations are thus required to evaluate the risks still posed by these chemicals.

Table 6 Alkylphenols (e.g., octylphenol)

Type or Source	Surfactant
Legal Status	Restricted for some uses
NRs (Mode of action)	ERs, AR, constitutive androstane receptor (CAR)
In vitro/Animal Studies	Proliferation of breast cancer cells; Resistin expression upregulation
Human Epidemiology Studies	
Developmental Exposure Studies	
Human Exposure	NP TDI: 7.5 mg/day
Levels in human body	In urine: range of 0.4–13.9 ng/mL; In adipose tissue: median level of 57 ng/g lipid
Biological half-life	NP in blood: 2–3 h
Concentration experimentally used	In vitro: lowest effect concentrations in the 10–1000-nM range

Adapted from: Casals-Casas, C., and Desvergne, B. (2011) Endocrine disruptors: from endocrine to metabolic disruption, Annu Rev Physiol 73, 135-162.

g. Bisphenol A



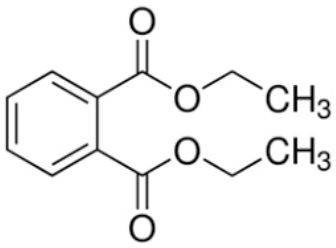
Bisphenol A (BPA) is a high-volume-production monomer used in polycarbonated plastic, in polystyrene resins, and as dental sealants. It is also used as an additive to other plastics such as PVC, and halogenated derivatives of BPA are widely used as flame retardants. Because unbound monomers remain after BPA polymerization, BPA molecules can be released from beverage and food containers, for example, from plastic baby bottles or from tin can liners. Human exposure to BPA is thus widespread, and unconjugated BPA molecules are detected in human blood, tissues, and milk. In a reference study in the United States, as many as 95% of human urine samples contained detectable levels of BPA in a range that is predicted to be biologically active. Estrogenic properties of BPA were first described in 1936. Since then, experiments performed in rodents have confirmed its hormonal activity, although the models and the high doses reported do not allow direct transposition to human risks. Thus, the potential human health risks caused by BPA exposure remain fiercely debated. Experimental data have been used to evaluate long-term exposure of mammalian model organisms during development and in adulthood to low doses of BPA (levels that fall below the regulatory safety standard). In short, these studies point to a number of adverse effects in mammals that include abnormal penile/urethra development, decreased sperm count, early sexual maturation in females, and brain and behavioral abnormalities. As such, the potential impact of BPA on human health is not easily dismissed. A few epidemiological and preliminary studies, based on small populations, have uncovered associations between BPA blood levels in women and various ailments, including obesity, recurrent miscarriages, and sterility. Additionally, higher urinary concentrations of BPA are associated with an increased prevalence of cardiovascular disease, diabetes, and liver enzyme abnormalities. Canada was the first country to ban the use of BPA in baby bottles.

Table 7 Bisphenol (BPA)

Type or Source	Plasticizer
Legal Status	Canada becomes the first country to ban BPA in baby bottles
NRs (Mode of action)	ERs, AR, TR, Glucocorticoid receptor (GR)
In vitro/Animal Studies	Reproductive effects; Adipogenesis induction, insulin increase
Human Epidemiology Studies	Potential reproductive effects. Associated with diabetes and liver abnormalities
Developmental Exposure Studies	Increased body weight (mice and rats)
Human Exposure	TDI: <50 µg/kg
Levels in human body	Range of 0.1–10 ng/ml in blood, urine, fat, and fetal tissue
Biological half-life	6 hr
Concentration experimentally used	In vitro: lowest effect concentrations in the 0.1–1-nM range; In mice: weight increase correlated with in utero exposure to 2.4–500 µg/kg/day.

Adapted from: Casals-Casas, C., and Desvergne, B. (2011) Endocrine disruptors: from endocrine to metabolic disruption, Annu Rev Physiol 73, 135-162.

h. Phthalates



Phthalate esters have been used worldwide as softeners to impart flexibility, pliability, and elasticity to otherwise rigid polymers such as PVC. Produced in large quantities since the 1930s, nearly all groups of industrial consumer products contain phthalates or traces of phthalates. These molecules are found mostly in industrial paints and solvents but also in toys, personal-care products, and medical devices such as intravenous tubing and blood transfusion bags. In such devices, they can make up 80% of the product's weight. Unlike BPA, phthalates are not covalently bound to the polymer matrix, making them highly susceptible to leaching. As a result, phthalates contaminate food, particularly meat and milk products, and are found nearly everywhere in interior environments. In addition, important routes of human exposure include dermal uptake from personal-care products and from plastic medical devices that come into direct contact with biological fluids. Exposure to phthalates can occur in the developing fetus through the placenta-blood barrier and in postnatal stages during breast feeding or from mouthing toys and baby-care products. Once incorporated into the human body, phthalates are shortlived and are rapidly metabolized.

Table 8 Phthalates (i.e. DEHP)

Type or Source	Plasticizer
Legal Status	Restricted in children's toys
NRs (Mode of action)	PPARs, CAR/PXR, GR
In vitro/Animal Studies	Cancer (liver, testes); Reproductive effects; Adipogenesis induction in cells, body weight decrease in mice
Human Epidemiology Studies	Potential reproductive and developmental effects. Associated with obesity and insulin resistance
Developmental Exposure Studies	DiBP: reduced plasma insulin and leptin levels in mice
Human Exposure	DBP's TDI: 10 mg/kg/day
Levels in human body	Range of prenatal phthalate metabolite mean levels in urine of mothers: 2.54–816 µg/L. Monoesters of DEHP in children's urine: 91.3 µg/L
Biological half-life	From hours to days
Concentration experimentally used	In cells: 50-µM DEHP, DBP, and metabolites

Adapted from: Casals-Casas, C., and Desvergne, B. (2011) Endocrine disruptors: from endocrine to metabolic disruption, Annu Rev Physiol 73, 135-162.

Among all the phthalates, diethylhexyl phthalate (DEHP) elicits the most concern, with more than two million tons produced annually. This compound is widely used in medical devices and in a variety of food products. DEHP causes animal toxicity in many physiological systems; however, many of the abnormalities that have been characterized since the 1940s have occurred at high DEHP doses. In addition, DEHP promotes liver tumor development in rodent models through severe peroxisomal proliferation. However, peroxisome proliferation has not been observed in humans, and according to a decision of the International Agency for Research on Cancer, DEHP cannot be classified as a human carcinogen. Experimental studies at low doses of DEHP exposure, which appear to be most pertinent to human health, have demonstrated subtle reproductive toxicity in male rodents. Other reproductive outcomes include testicular dysgenesis together with permanent feminization and demasculinization, resulting in a reduced anogenital distance.

Some epidemiological studies reported an association between cord blood levels of mono(2-ethylhexyl) phthalate (MEHP), a DEHP metabolite, and shorter gestational age of delivery. Indirect evidence also suggests that diethyl phthalate and dibutyl phthalate may impart antiandrogenic effects in the perinatal period. Maternal urine levels of metabolites of DEHP (benzylbutyl phthalate, diethyl phthalate, and dibutyl phthalate) are associated with a higher risk of incomplete testicular descent for male human infants and are inversely correlated with the anogenital distance. Other developmental effects of phthalate exposure may cause damage to the pulmonary system and may result in asthma. More recently, several studies have demonstrated a correlation between phthalates and metabolic disorders. In short- and long-term rodent studies, dose-related deregulation of levels of serum insulin, blood glucose, liver glycogen, T3, T4, thyroid-stimulating hormone, and cortisol was observed. In humans, the log-transformed concentrations of several phthalate metabolites positively correlated with abdominal obesity and insulin resistance in adult males. These analyses support the concept of environmental obesogens but await further confirmation by longitudinal studies.

4. Endocrine and metabolic disruption: Mechanistic approaches

Hormones in general and sex steroid hormones in particular contribute to both endocrine effects as well as general body homeostasis through diverse metabolic regulations. Thus, a certain number of metabolic perturbations are simply the result of hormonal disruption. Conversely, direct EDC activity through receptors responding to xenobiotics and regulating xenobiotic metabolism may also contribute to a metabolic phenotype as well as alter the endocrine response. We will discuss the mechanisms by which the xenobiotics discussed above affect the endocrine and the metabolic systems, leading to the toxicities listed in Table 1-8. (See Figure 3).

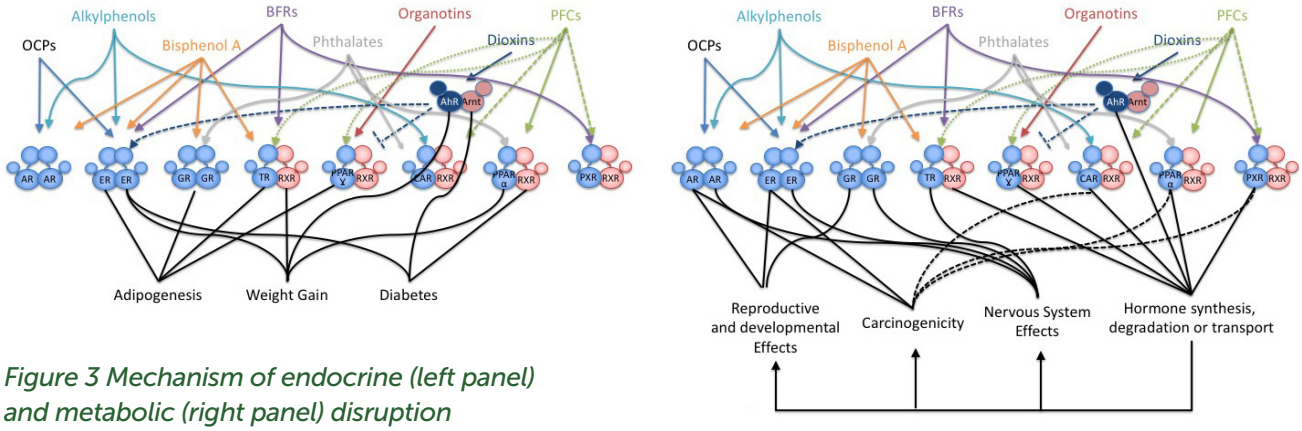


Figure 3 Mechanism of endocrine (left panel) and metabolic (right panel) disruption

Classic hormone receptors that recognize only one or a few molecules with high affinity. Thyroid hormone (TH), mineralocorticoid, glucocorticoid, retinoic acid, estrogen, vitamin D, progesterone, and androgen receptors belong to this class. Initial studies identified estrogen receptor α and β (ERα, ERβ) and androgen receptor (AR) as the targets of many EDCs, which resulted in developmental and reproductive effects, as well as metabolic alterations. There is increasing evidence that these classic hormone receptors are linking to central nervous system and behavior as well.

a. Metabolic disruption mediated by inappropriate activation of the estrogen receptor.

ERα and ERβ are the main mediators of the biological effects of estrogens. Upon estrogen binding, they form homodimers that bind to the promoters of estrogen-responsive genes. In addition to their well-established roles in reproduction, ERα and ERβ are involved in brain development and function of many other organs, such as skin, bone, and liver. Several lines of evidence link ERs to metabolism. For example, in postmenopausal women and ovarectomized rodents in which estrogen is low, one observes an increase in white adipose tissue; estrogen replacement therapy reverses these effects. ERα but not ERβ appears to mediate these effects, as inferred from studies using mice in which ERα is knocked out: Both male and female mutant mice show increased insulin resistance and impaired glucose tolerance. At the molecular level, ERs and estrogens regulate many aspects of metabolism, including glucose transport, glycolysis, mitochondrial structure and activity, and fatty acid oxidation.

A major question concerns exposure to estrogenic EDCs during the critical period of development. Indeed, embryos and fetuses are likely to be much more sensitive to perturbation by endocrine-like activities. Protective mechanisms available in adult animals, such as DNA repair mechanisms or liver detoxification and metabolism, are not fully functional in the fetus or neonate. Thus, exposure to EDCs during this period can cause adverse effects, some of which are not apparent until much later in life. This point is best illustrated by prenatal exposure to the estrogen derivative diethylstilbestrol (DES), which was widely used until the 1970s as an antimiscarriage medication; this early exposure impaired reproduction later in life. With regard to EDCs, the effects of prenatal exposure to BPA are well documented. In contrast to the reduced body weight associated with BPA exposure in adult rodents, exposure to BPA during fetal life resulted in an increase in adult body weight. In rats, perinatal exposure to low BPA doses increased adipogenesis and body weight in adult females, which exhibited adipocyte hypertrophy.

b. Metabolic disruption through inappropriate activation of thyroid hormone receptor and glucocorticoid receptor.

EDCs may also modulate other hormone nuclear receptors, particularly thyroid hormone receptor (TR) and glucocorticoid receptor (GR). Most TH activity is mediated by the TRs TRα and TRβ, which form heterodimers with RXR to bind the promoter sequences of target genes. TR agonists relieve the repression that unliganded TRs may exert on some target genes, thus further inducing gene expression. In addition to an important role in brain development, THs are tightly associated with metabolism. Elevated TH levels accelerate metabolism, increase lipolysis as well as hepatic cholesterol biosynthesis and excretion, and provoke weight loss. The exact opposite results are observed with low TH levels.

In contrast to TR, GR forms homodimers and resides in the cytosol, forming complexes with molecular chaperones. Ligand binding releases the chaperones, triggers GR nuclear translocation, and influences gene expression. Glucocorticoids acting through GRs allow an organism to adequately respond to physical or emotional stresses by promoting gluconeogenesis, increasing blood glucose levels, and mobilizing the oxidation of fatty acids. The pharmacological uses of glucocorticoids, chiefly in the context of controlling chronic inflammation, have serious metabolic side effects such as diabetes, muscle wasting, and growth retardation in children.

EDCs also interact with these TR and GR receptors. For instance, in differentiating 3T3- L1 cells, BPA and dicyclohexyl phthalate stimulate GR-mediated lipid accumulation and synergize with a weak GR agonist to increase expression of adipocyte-specific markers. BPA may also act as an antagonist of the TR pathway by enhancing recruitment of the corepressor NCoR to TR. In parallel, perinatal exposure of BPA increases levels of thyroxine (T4). Given the important role of TH in energy homeostasis, BPA effects on TR during development may be important in longterm body weight increase. BFRs also disrupt the TH pathway, and daily exposure of rats to PBDE over four weeks resulted in a significant increase in lipolysis and a significant decrease in glucose oxidation, characteristics associated with obesity, insulin resistance, and type 2 diabetes, although such exposure had no effect on body weight and adipocyte size.

c. Endocrine and Metabolic Disruption Through Xenosensors

The body is protected from the accumulation of toxic chemicals by a complex strategy that in part takes place in the liver, regulating the expression of drug-metabolizing enzymes and transporters. This adaptive response incorporates at least three xenosensors: pregnane X receptor (PXR), constitutive androstane receptor (CAR), and aryl hydrocarbon receptor (AhR).

i. Pregnane X receptor and constitutive androstane receptor.

PXR and CAR are members of the NR superfamily of sensor receptors, and although they were originally defined as xenosensors involved in regulating the metabolism of xenobiotics, their contribution to fatty acid, lipid, and glucose metabolism has been only recently appreciated. PXR and CAR regulate gene expression by forming heterodimers with RXR that bind to xenobiotic response sequences present in the promoters of their target genes. However, their mechanisms of activation differ.

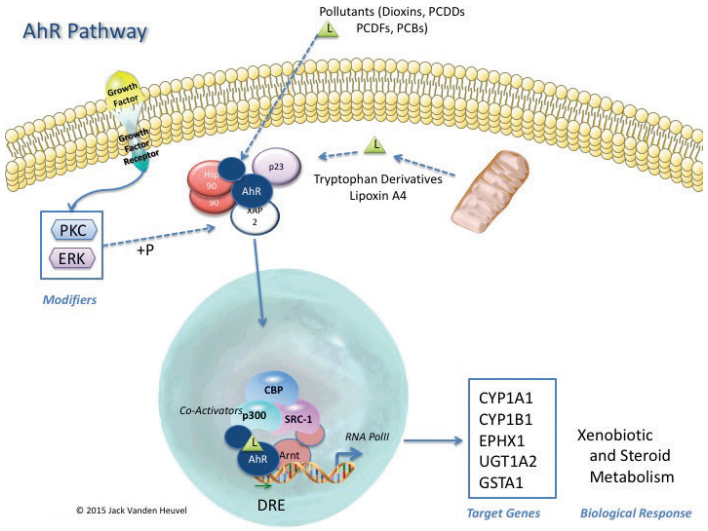


Figure 4 Mechanism of action of AhR

PXR is located primarily in the nucleus and is strongly activated upon ligand binding. In contrast, in the absence of ligand, CAR is retained in the cytoplasm through association with the cytoplasmic CAR-retention protein (CCRP) and heatshock protein 90 (HSP90). In the presence of activators, CAR dissociates from its two chaperones and translocates into the nucleus, where it forms heterodimers with RXR. PXR and CAR are highly expressed in the liver, where they act as master regulators of detoxification pathways through induction of phase I to phase III enzymes. In the first phase, a polar group is added to hydrophobic substrates by hydroxylation and oxidation via the cytochrome P450 (CYP) mono-oxygenase system. CYP3A is responsible for the metabolism of up to 60% of the drugs presently on the market and is a major target gene of PXR, whereas phenobarbital-induced activation of CAR triggers the expression of CYP2B. Phase II enzymes increase hydrophilicity of the compounds through various conjugation reactions, and phase III involves transporters that allow for removal of these compounds through secretion. Endogenous ligands for PXR and CAR include bile acids and their metabolites. A number of xenobiotics affect both these NRs, which supports their role xenosensors involved in a coordinated adaptive response. They recognize an increase in steroid-like chemicals and, in turn, induce detoxification. Furthermore, PXR and CAR induce enzymes, such as the CYP2B and CYP3A family members, responsible for the metabolism of steroid and thyroid hormones and this may alter their normal physiological function.

A number of EDCs activate PXR and CAR; both may be activated by nonylphenol, DEHP, and MEHP. BPA and some PCBs activate human PXR, whereas PFOA, PFOS, and the organochlorine methoxychlor can activate CAR. As mentioned above, PXR and CAR were identified chiefly as xenobiotic-metabolizing regulators; however, clinical observations revealed that many CAR and PXR activators affect lipid and glucose metabolism in patients. For instance,

the known PXR activator rifampicin induced liver steatosis in tuberculosis patients, and long-term treatment with phenobarbital provoked significant changes in hepatic and plasma metabolite profiles. Furthermore, laboratory animal and in vitro studies show a similar trend: PXR activation induced a steatogenic effect in rat and mouse liver, and CAR and PXR activators repressed hepatic gluconeogenic enzymes and genes. CAR was recently described as an antiobesity NR that ameliorates diabetes and fatty liver.

ii. Aryl hydrocarbon receptor

AhR is a ligand-activated transcription factor that belongs to the basic helix-loop-helix Per- ARNT-SIM (bHLH-PAS—where Per denotes the Drosophila melanogaster clock gene Period; ARNT denotes aryl hydrocarbon receptor nuclear translocator; and SIM denotes a neurodevelopmental regulator in flies, single-minded) family of proteins. AhR is a xenosensor that mediates the biological response to a wide spectrum of xenobiotics; in particular, AhR is the major factor sensing and mediating the toxic effects of the dioxin TCDD.

The nonactivated AhR protein resides in the cytosol and, upon ligand-mediated activation, translocates into the nucleus, where it heterodimerizes with the ubiquitously expressed ARNT, a member of the same protein family (see Figure 4). The AhR/ARNT complex binds to specific regulatory DNA sequences to regulate gene expression. AhR activity may also be mediated by alternative ligands and by an ARNT independent mechanism, although details of these mechanisms remain poorly understood.

Among the targets involved in detoxification, AhR target genes include the phase I enzyme CYP1A1 and the phase II enzymes UGT1A1 and UGT1A6. In addition, AhR may contribute to the coordinated regulation of human drug-metabolizing enzymes and conjugate transporters by inducing PXR and CAR expression. Endogenous molecules that bind AhR and benefit from detoxification activity are lipoxin 4 and leukotriene derivatives, as well as the heme metabolites biliverdin and bilirubin.

Xenobiotics that activate AhR include various dietary phytochemicals, some PCBs, and TCDD. Because it is very poorly metabolized, TCDD triggers sustained activation of AhR, contributing to the toxic effects of dioxin. These toxic effects thereby highlight the undesired events that may occur through inappropriate AhR activation and reveal a subset of AhR target genes unrelated to detoxification. These targets include the CDK inhibitors p21CIP1 and p27Kip1, which may explain the broad role of AhR in organogenesis, embryonic development, the cell cycle, immunosuppression, and carcinogenicity.

Recently, AhR has been implicated as a regulator of energy metabolism. Epidemiological studies show an association between dioxin exposure and type 2 diabetes. Other studies also demonstrate that high and low doses of dioxins affect genes in an AhR-dependent manner linked with hepatic circadian rhythm, cholesterol biosynthesis, fatty acid synthesis, glucose metabolism, and adipocyte differentiation. The mechanisms by which AhR regulates energy metabolism are not yet well described, but various direct and indirect mechanisms including cross-talk with ER may be involved. AhR may disrupt the ER signaling pathways through increased ER proteasomal degradation, modulating estrogen levels via CYP expression, altering ER transcriptional activity via coactivator squelching, or promoting DNA-binding competition. In addition, AhR also indirectly affects adipogenesis through inhibition of PPARγ expression. Additional experimental and epidemiological studies are still required to assess whether AhR-mediated responses affect metabolism in addition to the well-known roles of Ahr in immunity, development, and cancer.

c. Metabolic Disruption Through Peroxisome Proliferator–Activated Receptors

Metabolic homeostasis requires a controlled balance between energy storage and consumption; several NRs and their coregulators are instrumental in these processes. Among these, the PPARs act as lipid sensors that cooperate in different organs to adapt gene expression to a given metabolic status. PPARs are sensor receptors with a rather large ligand-binding domain, which can accommodate a variety of ligands, primarily lipid derivatives. In the presence of ligand, PPARs heterodimerize with RXR and bind to the PPAR response elements localized in the promoter regions of their target genes. The PPAR family is composed of three isotypes: PPARα, -β/δ, and -γ. PPARα is expressed predominantly in tissues characterized by a high rate of fatty acid catabolism such as liver, kidney, heart, and muscle. PPARα was first identified as the protein responsible for the induction of peroxisome proliferation in rodents exposed to a variety of compounds collectively termed peroxisome proliferators. However, humans do not undergo peroxisome proliferation and are thereby protected from the consequent liver tumors observed in sensitive species. PPARα plays a major role in fatty acid oxidation in all species, controlling lipoprotein metabolism and limiting inflammation. PPARβ is ubiquitously expressed, shares partially overlapping functions with PPARα, and also plays a role in cell differentiation and survival. Finally, PPARγ functions in adipogenesis, lipid storage, and the control of insulin sensitivity; it also participates in inflammatory responses.

Plasticizers, surfactants and certain pesticides can modulate PPAR activity. The specificity of this PPAR-mediated response is highlighted in a study in which 200 pesticides were systematically screened for their peroxisome proliferation activity. Only three compounds were identified as having PPARα transcriptional activity, and none possessed PPARγ transcriptional activity. Among these pesticides, diclofop-methyl and pyrethrins induced PPARα target gene expression. The phthalates are another group of well characterized peroxisome proliferators. In vitro transactivation assays and intact cellular systems were used to reveal that phthalates and their metabolites bind and activate the three PPARs, among other NRs. These studies also determined the range of potency and efficacy of phthalate monoesters, showing differences between isotypes and species. Modeling the DEHP metabolite MEHP in the PPARγ ligand-binding pocket indicates that MEHP may contact residues similar to those defined for the classic PPARγ agonist rosiglitazone. MEHP induces adipogenesis in a PPARγ-dependent manner, albeit with lower efficiency than rosiglitazone in 3T3-L1 cells. MEHP acts as a selective modulator of PPARγ rather than as a full agonist. Taken together, these in vitro data demonstrate that MEHP is proadipogenic in a cell culture model, suggesting that it may act as a metabolic disruptor and may promote obesity in vivo.

The PFCs, particularly PFOA and PFOS, can also activate mouse and human PPARs in transactivation assays. Adult mice exposed to high doses of PFOA exhibit weight loss, which is abrogated in PPARα-null mice. The proposed mechanism involves PPARα-dependent anorexigenic activity in the hypothalamus of adult rodents. Developmental exposure to low PFC levels results in increased body weight and increased serum insulin and leptin levels at midlife. Again, species-specific PPARα activity was proposed because low doses of PFOA significantly activate the function of PPARα in wild-type mice but not in PPARα-humanized mice.

Several EDCs also specifically target PPARγ. Using a high-throughput screen, 40 EDCs, organotins such as TBT and TPTO are activators of human RXR and PPARγ. TBT binds to and activates the three human subtypes of RXR as well as many permissive heterodimeric partners such as liver X receptor (LXR), nuclear receptor–related 1 protein (NURR1), PPARβ, and PPARγ, but not PPARα. Organotins bind and activate, primarily through RXR and not through PPARγ, the PPARγ:RXR heterodimer at nanomolar concentrations. The crystal structure of the RXRα ligand-binding domain bound to TBT indicates that TBT binds with high affinity to RXR, even though TBT is structurally distinct from above-described ligands and only partly occupies the RXRα ligand-binding pocket.

Consistent with the critical role played by PPARγ:RXR signaling in mammalian adipogenesis, TBT promotes adipogenesis in 3T3-L1 cells by direct transcriptional effects on the PPARγ target genes. In utero exposure to TBT in rodents led to alterations in fat structure and metabolism, with a disorganization of hepatic and gonadal architecture, steatosis in the liver, and an increase in lipid accumulation and mature adipocytes. The fat mass—but not the total body weight—of in utero TBT-treated mice significantly increases in adulthood, supporting the conclusion that embryonic and chronic lifetime organotin exposure may contribute to the incidence of obesity through disruption of the PPARγ:RXR pathway.

5. Conclusions

Altogether, these many examples of EDC interaction with receptors highlight the fact that a given compound can interfere with different NRs and different pathways (see Tables 1-8). For example, depending on the compound, BFRs interface with AR, ER, and progesterone receptor to elicit both agonist- and antagonist-like effects. PBDEs bind but do not activate AhR; in contrast, they induce the expression of various CYP enzymes, in part through the activation of PXR PBDEs are also active in TH regulation by disrupting peripheral TH transport and metabolism/deactivation or by binding and activating TRs. The final consequences of EDCs exposure are thus due to cross-talk between these pathways, rather than to a linear causation chain and are much more complex to decipher in vivo.

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