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Endocrine Disruptors: Focus on the Adrenal Cortex

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ABSTRACT

Endocrine-disrupting chemicals (EDCs) are exogenous substances known to interfere with endocrine homeostasis and promote adverse health outcomes. Their impact on the adrenal cortex, corticosteroids and their physiological role in the organism has not yet been sufficiently elucidated. In this review, we collect experimental and epidemiological evidence on adrenal disruption by relevant endocrine disruptors. In vitro data suggest significant alterations of gene expression, cell signalling, steroid production, steroid distribution, and action. Additionally, morphological studies revealed disturbances in tissue organization and development, local inflammation, and zone-specific hyperplasia. Finally, endocrine circuits, such as the hypothalamic-pituitary-adrenal axis, might be affected by EDCs. Many questions regarding the detection of steroidogenesis disruption and the effects of combined toxicity remain unanswered. Not only due to the diverse mode of action of adrenal steroids and their implication in many common diseases, there is no doubt that further research on endocrine disruption of the adrenocortical system is needed.

Introduction

In times of the Anthropocene, increasing production and environmental release of synthetic chemicals pose a significant challenge for ecological balance and a hazard to human health. The planetary resilience for anthropogenic pollution in terms of quantity and diversity of chemicals introduced and distributed is postulated to be already reached [1]. By today, approximately 140 000 chemical substances have been registered, of which 5000 are extensively produced and dispersed. Less than half have undergone adequate environmental risk assessment, as testing has only become mandatory in recent decades, particularly in high-income countries [2, 3]. A relevant proportion of these substances are able to interfere with cellular structures of endocrine organs and alter endocrine pathways or homeostasis. Such endocrine-disrupting chemicals (EDCs) might be defined as "exogenous substance(s) or mixture that (alter) function(s) of the endocrine system and consequently (cause) adverse effects in an intact organism, or its progeny, or (sub-)populations" [International Programme on Chemical Safety (IPCS)] [4].

EDCs can be found in agricultural, industrial, domestic, and medical applications. They have been or are used as herbicides or fungicides in food production [e. g., dichlorodiphenyltrichloroethane (DDT), atrazine, glyphosate], as plasticisers (e. g., phthalates, bisphenols), in personal-care products (e. g., parabens, benzophenones) [5], and in construction, furniture, and electrical ap-

plications as flame retardants [e. g., polybrominated diphenyl ethers (PBDE)]. Ultimately, they are found in children's toys (e. g., phthalates, PBDE) [6] 2,3,7,8-Tetrachlorodibenzo-p-dioxin. Dioxins emerge as by-products of industrial combustion (e. g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)). Polychlorinated biphenyls (PCBs) have been used in capacitors, while per- and polyfluorinated substances (PFAS) serve in water-repellent textiles or in fire-fighting foams. Some heavy metals, such as cadmium, copper, or mercury, increasingly mined and distributed, count among the list of endocrine-disrupting substances. Micro- and nanoplastic particles as products of physical and environmental degradation can serve as hydrophobic absorbing surfaces for EDCs with whom they can enter organisms and cross epithelial barriers [7]. The mentioned substances rank among the most prominent disruptive agents, which, at the same time, we encounter on a daily basis [8].

From their primary fields of application, these substances are released through volatilization. By now, they have been distributed around the globe and found even in remote regions, such as the Mount Everest [9] or the Mariana Trench [10]. Global transport of toxins via air and ocean currents, global (waste) trade and supply chains make the distribution of potential hazardous chemicals a worldwide problem [11]. For instance, ubiquitous particulate matter (PM) delivers complex mixtures of air pollutants, such as nitric or sulfur oxides, heavy metals, or polycyclic aromatic hydrocarbons, which are known for an endocrine disruptive activity [12]. Despite ambitious political regulations, restricted chemicals can and will still be found in recycled materials for a long time complicating their elimination from environmental cycles [6, 13].

In addition, some endocrine-disruptive agents are predisposed to biomagnification, accumulation, and long-range transport. Their long half-lifes indicate only little biodegradation and promote the enrichment in food chains and the environment [2]. For instance, if PBDEs were banned immediately, they would still be detectable in landfills beyond 2080 [14]. Widespread PFAS, however, appear to be highly resistant to environmental degradation allowing persistence for hundreds of years after release. Their thermal and chemical stability contribute to accumulating concentrations and ubiquitous exposure [15].

Finally, after incorporation via ingestion, inhalation or dermal uptake, EDCs can be detected in human samples, such as serum, urine, milk, but also placental tissue [16], seminal plasma [17], saliva [18], or amniotic fluid [19]. Individual levels might depend on lifestyle, profession, socioeconomic status [20], local environment, and political regulations. Regional initiatives conduct systematic biomonitoring studies allowing the analysis in different populations, age groups and countries (e. g., HBM4EU [21]). Nevertheless, all humans are certainly continuously exposed to a complex mixture of low-dosed EDCs with different structures and toxicokinetic properties [8].

The tremendous amount and variety of introduced chemicals requires precise regulation and consideration of potential hazards for environmental balances and health of wildlife and humans [2]. Meanwhile, EDC-associated adverse outcomes resulted in ambitious regulation of substances, for example, via continuous determination of tolerated intake doses (TDI). For instance, just very recently the EFSA (European Food Safety Authority) proposed a TDI of 0.2 ng/kg body weight/day for bisphenol A (BPA) illustrating the need for continuous research of potential health implications in order to control exposure to hazardous substances [22]. Another milestone towards chemical safety is the Stockholm Convention on persistent organic pollutants banning most toxic substances in 2004 [23]. However, substitutes for hazardous endocrine disruptors, which are introduced in order to replace their precursor like bisphenol F (BPF) instead of BPA or DINCH (1,2-cyclohexanedicarboxylic acid diisononyl ester) replacing diethylhexyl phthalate (DEHP) are only superficially characterized. Retrospective hazard identification after years of unlimited use and release is a hazardous and unsustainable, yet common practice.

More and more epidemiological data show associations between EDCs and susceptibility for metabolic diseases (e. g., insulin metabolism, obesity), reproductive impairments (e. g., reduced fertility, preterm births, polycystic ovary syndrome, or cryptorchidism), incidence of hormone-related cancers (e. g., breast and prostate cancer), but also neurocognitive and behavioural pathologies. Evidence of disruption of the thyroid system (e. g., altered T3/TSH levels, iodine uptake) is growing, as well as the potential impairment of hypothalamic-pituitary axes [8]. Besides, EDCs might be risk factors for foetal development. Crossing placental barriers, they might contribute to an unfavourable environment in utero [24].

While preliminary data from observational studies indicate an association with adrenal neoplasia [25, 26], no causality between the exposome (sum of environmental factors influencing human health) and the development of disorders of the adrenal cortex, associated hormones and their circadian rhythmicity has been described, yet. Data on the interaction of EDC and the adrenal gland are still scarce compared to other endocrine glands, such as the thyroid. We therefore try to shed light on this important aspect of endocrine physiology and pathophysiology.

The adrenal gland as toxicological target

The adrenal gland consists of the cortex and the medulla. Steroidogenesis in adrenocortical cells is mediated by a cascade of enzymes. Cells of the subcapsular zona glomerulosa (ZG) produce mineralocorticoids, maintaining sodium/potassium homeostasis. Cells of the zona fasciculata (ZF) produce glucocorticoids, especially cortisol, relevant for stress response, metabolic homeostasis, and immune regulation. Cells of the reticular zone (ZR) produce precursors of androgens, for example, dehydroepiandrosterone (sulfate) and androstenedione. Feedback loops between limbic areas, the hypothalamic paraventricular nucleus (PVN), pituitary and adrenal glands allow the maintenance of precise steroid levels under physiological conditions [27].

Nevertheless, the exposure of the adrenal tissue to endocrine disruptive agents cannot be quantified sufficiently in vivo [26]. Conventional biomonitoring of body fluids, for example urine, does not allow to draw clear conclusions on adrenal concentrations of EDCs. However, the adrenal cortex has several predisposing characteristics making it particularly vulnerable to endocrine disruption [27, 28].

The efficient blood circulation enables the adrenal gland to rapidly release its products into the bloodstream, but, on the other

hand, contributes to the constant exposure to potential disruptive substances. Additionally, the lipophilic milieu due to the high content of cholesterol esters in the adrenal cortex enables lipophilic toxicants to accumulate and endure. The high density of receptors such as high-density lipoprotein (HDL) and scavenger receptor class B type 1 (SRB1) for the uptake of lipid precursors allow the storage and persistence of lipophilic contaminants. For instance, flame-retardant PBDEs were shown to be effectively absorbed after oral administration, retained in lipophilic tissue and enriched in certain organs, such as the adrenal glands [29, 30]. Additionally, derivatives of the insecticide DDT were found to accumulate and to be activated in the adrenal ZF in mice [31], again suggesting the adrenal cortex to be predisposed for the influence of at least some EDCs in vivo.

The fine-tuned functionality of cytochrome P450 (CYP) enzymes in the adrenal gland carries the potential to metabolise and further activate exogenous substances, potentially aggravating their local toxicity. For instance, DDT-metabolites were covalently bound to proteins and water-soluble derivates were formed in vitro, which was inhibited by CYP-inhibiting metyrapone [31]. Besides, the adrenal's susceptibility for oxidative stress-induced lipid peroxidation is generally elevated due to the abundance of polyunsaturated fatty acids in adrenal lipid membranes.

Importantly, the adrenal gland is an important player in endocrine circuits, like the hypothalamic-pituitary-adrenal axis (HPA) or the renin-angiotensin-aldosterone system (RAAS). Therefore, disruption of endocrine processes in the brain or the kidney might affect adrenal homeostasis as well. Last but not least, EDCs might target multiple extra-adrenal systems involved in steroid distribution or peripheral hormone action, for example, by competitive binding of hormone carrier proteins, (ant-)agonism at target receptors, and peripheral (de-)activating of enzymes [27, 32].

Key characteristics for the determination of endocrine-disruptive properties of chemicals were identified in a consensus statement for improved hazard identification [33]:

- (ant-)agonism at cellular receptors
- alteration of receptor expression
- interference with intracellular signalling pathways
- interaction with nucleic acids via epigenetic modifications
- alteration of hormone synthesis, secretion, and distribution
- effects on proliferation, differentiation, or migration
- interference with cell fate and tissue organisation
- effects on peripheral enzyme and receptor expression [34],
- disruption of circadian rhythmicity in hormone secretion and gene expression [35],
- induction of oxidative stress [36–37]

Common endocrine disrupting chemicals, their primary application, and experimental outcomes regarding the adrenal cortex are listed in ▶ **Table 1**.

Agonism/Antagonism at receptors

EDCs act as exogenous ligands at nuclear receptors. In steroid-producing cells, these include estrogen (ERα/β), androgen (AR), glucocorticoid (GR), mineralocorticoid (MR), and progesterone (PR)

receptors. EDCs' effect on nuclear receptors is mediated by direct binding, impairment of nuclear translocation, binding to hormone-responsive elements or via changes in receptor expression [38].

For instance, BPA's affinity to ERs and its estrogenic effects have been reported already in 1936. Further, next-generation bisphenols show similar actions at ERs. Meanwhile, computational analyses identified distinct binding sites of bisphenols on several steroid receptors [39], while receptor activation was confirmed by reporter gene assays in vitro [40–42].
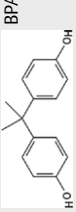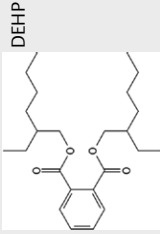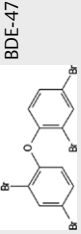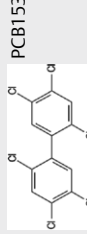
Some high-weight phthalates show higher affinity to AR, PR, and GR than endogenous ligands [43] and might thereby mediate their action on the adrenal gland in vivo [44]. For instance, DEHP and monoisodecyl phthalate (MIDP) showed stronger binding to GR than the natural ligand cortisol in silico [43]. The fungicide atrazine has been shown to interact with steroidogenic-factor 1 (SF-1/NR5A1), a nuclear receptor regulating steroidogenic pathways, followed by an activated signalling and enhanced transcription of steroidogenic enzymes in vitro [45]. Peroxisome proliferator agonist receptor (PPAR) subtypes serve as regulators of genes involved in lipid metabolism or inflammatory processes in the adrenal cortex. For instance, substances, that are introduced as alternatives to hazardous phthalates, such as DINCH, bind and activate PPARγ. Para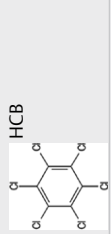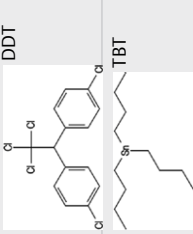llel lipid accumulation and provoked oxidative stress in adipocytes is suggested to be partly mediated by this receptor interaction [46].

Zhang et al. mapped the endocrine activity of contaminated soil samples on steroid-dependent nuclear receptors, such as AR, MR, or GR. Most samples contained receptor-binding compounds, such as DDT, and other chlorinated contaminants underlining the problem of pollution with endocrine active substances [47].

Receptor expression

Modifying receptor expression in the adrenal gland and responsiveness to physiological stimuli, especially adrenocorticotropic hormone (ACTH), angiotensin II (AngII), or potassium, represents another potential target of EDCs. Physiological ligand-receptor interactions and subsequent signalling may be affected by dysregulated receptor density. The melanocortin receptor 2 (MCR2), responsible for ACTH signalling in the ZG and ZF, is altered by perfluorinated octanoic acid (PFOS) and PCB126 on the mRNA level [48]. Transcriptomic analyses following prenatal diethylhexyl phthalate (DEHP) treatment of rats revealed altered gene expression of potassium channels and AngII receptors: KCNK5 expression was upregulated [49], whereas the angiotensin II receptors AGTR1a/b were downregulated, followed by altered aldosterone secretion [50]. SF-1 expression was shown to be enhanced by 4-bromodiphenyl ether (BDE-3) in vivo [51]. Moreover, alterations of receptor activity involved in cholesterol metabolism have been shown to be affected by EDCs. While the low-density lipoprotein (LDL) receptor was enhanced by DEHP [50], the preferred pathway for cholesterol uptake via SR-B1 was upregulated by BDE-3 [51].

▶ Table 1 Common endocrine disrupting chemicals, their primary application, and experimental outcomes regarding the adrenal cortex. Arrows indicate increasing or decreasing effects. Formulas were drawn with PubChemSketcher V2.4.

Endocrine-disrupting chemicals		Structure	Application	Exemplary outcomes [Ref]
Bisphenols	Bisphenol A (BPA), Bisphenol F (BPF)	BPA	Plasticizers in polycarbonate plastics, epoxy and vinyl resins; found in plastic bottles, baby bottles, thermal and currency paper, coatings in drinks and food cans	Estrogen, gluco-/mineralocorticoid, progestogen, androgen activity [39–42]; disrupted gene expression of StAR [58], CYP, HSD [63, 64]; ↓ antioxidant defence, ↑ ROS [36]; ↑ serum glucocorticoids (GC) [74, 75]; ↓ GR expression [75]; vascular congestion [36]; ↑ ZF thickness, ↓ ZR thickness [75]; less robust coping to stressors, sex-dependent neurobehavioral alterations (anxiety, depression-like phenotype) [73–74]
Phthalates	Diethylhexyl-phthalate (DEHP), Dibutyl-phthalate (DBP)	DEHP	Plasticizers in polyvinyl chloride polymers (PVC); found in wires, plastic bottles, medical equipment, lacquers, varnishes, paints, fixatives in perfumes, insect repellents	Androgen, glucocorticoid, progestogen activity [43]; PPARγ affinity [46]; disrupted expression of potassium channels [49], AGTR1a/b, LDL-R [50]; differentially activated transcription factors [54]; ↑ DNA methylation [44]; cholesterol synthesis [50]; disrupted expression of StAR, CYP, HSD [60–62]; CYP-inhibiting effects [65]; binding of CBG, SHBG [77, 78], human serum albumin [81]; ↑ ROS, gene and protein oxidation [60]; lipid accumulation [50]; ↑ cytokines [84]; angiectasis [84]; ↓ RAAS signalling [93]
Poly-brominated diethyl ethers (PBDE)	4-Bromodiphenyl ether (BDE-3), 2,2′-4,4′-Tetrabromo-diphenyl ether (BDE-47)	BDE-47	Flame-retardant substances; found in furniture, textiles, vehicles, plastics	↑ Cortisol, ↑ aldosterone, ↑ serum GC [59]; disrupted gene expression of StAR, CYP, HSD [51, 59], SF-1 [51]; altered cholesterol uptake, intracellular signalling [51]; selective adrenal weight gain [59]; accumulation in the adrenal cortex [29–30]
Poly-chlorinated biphenyls (PCB)	PCB126, PCB153	PCB153	Plasticizers, additives in PVC polymers, pigments in ink; industrial use as insulating fluids, for transformers, capacitors; hydraulic and lubricating fluids	↑ 17-OH-pregnenolone, ↑ DHEA; ↓ estradiol, ↓ corticosterone; disrupted gene expression of StAR, CYP, HSD [48]; elevated levels in aldosterone-producing adenoma [26]
Per- and poly-fluoroalkyl substances (PFAS)	Perfluoro-octane-sulfonic acid (PFOS)	PFOS	Water-repellent textiles and paper products; fire-fighting foams, skiing wax, cosmetics, impregnation agents	Disrupted gene expression of StAR, CYP, HSD, MC2R ; ↑ 17-OH-pregnenolone, ↑ DHEA; ↓ cortisol, ↓ corticosterone, ↓ aldosterone [48]; ↓ serum GC, ↓ ACTH, ↓ CRH [70]; ↑ serum CBG [80]; ↓ central expression of CRHR, GR [70]
Alkylphenols	Octylphenol (OP), Nonylphenol (NP)	OP	Detergents, additives in fuels, lubricants; found in fragrances, tires, adhesives, coatings, carbonless copy paper, rubber products	↑ Serum GC, ↑ ACTH, ↑ CRH; hyperplasia, ↑ vascularisation, macrophage infiltration [72]
Organo-chlorides	Hexachlorohexane/-benzene (HCH/HCB)	HCB	Agricultural pesticides, former pharmaceutical use (lindane)	DHEA ↑ [48]; elevated levels in aldosterone-producing adenoma [26]
	Dichlorodiphenyl-trichloroethane (DDT)	DDT	Agricultural insecticide, former pharmaceutical use (malaria, typhus, leishmaniasis), mitotane (= DDT-derivate)	Developmental retardation and dysmorphic growth [85, 86]; accumulated in adrenal cortex [31]; metabolization by adrenal CYP enzymes [31]
Organotins	Tributyltin (TBT)	TBT	Biocidal anti-fouling paint in ships, vessels; disinfectants, fertilizer, wood/textile production, stabilizer in PVC; found in textiles, plastic polymers, seafood	↑ 17-OH-pregnenolone, ↑ progesterone, ↑ DHEA, ↑ 11-deoxycorticosterone, ↓ corticosterone [48]; ↑ serum GC, ↓ ACTH [71]; hyperplasia ZG, ZF; loss of architecture [69]; lipid accumulation, mononuclear, neutrophil, mast cell infiltration; apoptosis induction fibrosis [71]

Intracellular signalling pathways

Pathways mediating essential intracellular cascades can be affected by EDCs. There is sufficient evidence that biochemical activation, that is, via phosphorylation, and translocation of signalling proteins, second messengers or transcription factors are disrupted in their precise function in the cell's adaptation to stimuli.

In mice treated with flame-retardant BDE-3, disrupted phosphorylation patterns of transcription factors, such as cAMP response element-binding protein (CREB), and enzymes, like AMP-activated protein kinase (AMPK) and c-Jun N-terminal kinases (JNK), have been shown. Thus, BDE-3-deactivated AMPK and CREB could affect enzyme levels via modification of transcription [51]. BPA has been found to induce JNK phosphorylation, associated with elevated CYP11A1 activity and corticosterone production in adrenal cells and in vivo [52]. In addition, the expression of transcription factors, such as sonic hedgehog (Shh) and its nuclear translocation to binding sites, might be impaired by BPA-bound ERβ. This resulted in increased cyclin D transcription and a potentially disrupted cell cycle [53].

Whole-genome sequencing following prenatal DEHP exposure of rats revealed dysregulated genes of transcription factors (CREB, CREM, NR4A1, NR4A3), PPAR and MAPK (mitogen-activated protein kinase) pathways. In adult rats, the susceptibility for further hits targeting similar pathways, for instance PPAR-antagonists, was enhanced [54].

Epigenetic changes

Endocrine disruptors can interfere with the human epigenome via changes in DNA methylation, histone modification and expression of aberrant microRNAs (miRNA) [55]. These alterations by EDCs are ultimately passed on to the offspring via multi- or transgenerational inheritance [24, 56].

DNA methylation levels of nearly two million CpG dinucleotides were assessed after in utero exposure of rat adrenal glands to DEHP, and 972 differentially methylated CpGs were identified. Alterations in gene expression of specific adrenocortical genes, such as angiotensin receptors, potassium channels or steroidogenic enzymes, could only partially be explained by epigenetic changes, as no significant epigenetic modifications were found in their promoter region. However, differentially methylated CpG clusters were associated with gene loci programming for immune response, cell cycle and growth and tissue development. It was hypothesised that DEHP might activate the nuclear receptors PPARα and PPARβ, thereby inducing epigenetic modifications manifested in altered gene expression and steroid secretion [44]. However, specific epigenetic changes in the adrenal development and function are rarely investigated.

Steroidogenic enzyme expression

Activity and expression of steroidogenic enzymes can be affected by EDCs as backlog effects, or altered metabolization of precursor hormones might imbalance steroidogenic capacity. mRNA levels of enzymes responsible for endogenous cholesterol synthesis and conversion (HMG-CoA-reductase and -synthase) were upregulated following prenatal DEHP exposure, promoting cholesterol production [50]. Transcriptional regulation of mitochondrial steroido-

genic acute regulatory protein (StAR) was profoundly altered by treatment with glyphosate over 14 days in adult male rats. Reduced proteinkinase A (PKA) activity led to hypophosphorylation of CREB and StAR itself promoting impaired function and transcription of StAR [57]. Meanwhile, BPA increased protein levels of StAR dose-dependently in H295A cells. This effect might be ER-mediated [58].

Transcription and translation of subsequent steroidogenic enzymes, for example, CYP11, CYP17, CYP19, CYP21, HSD3B2, and HSD17B1 have been shown to be altered by PBDEs [51, 59], phthalates [60–62], bisphenols [63, 64], PFAS, or PCBs [48]. In silico analyses demonstrated direct inhibitory effects of phthalates on CYP450 enzymes involved in steroidogenesis via molecular docking. Some phthalate metabolites show even stronger affinity to CYP19A1 or CYP11B1 than therapeutical CYP-inhibitors, for example, exemestane or metyrapone [65]. Phthalates, alkylphenols and bisphenols show direct inhibition of sulfotransferase SULT2A1, which catalyses the sulfonation of DHEA to DHEAS, detectable by accumulating radio-labelled substrate PAPS (3′-phosphoadenosine-5′-phosphosulfate) in vitro [66].

The peripheral action of adrenal glucocorticoids is regulated by hydroxysteroid dehydrogenases (HSD). HSD11B1/2 catalyse the conversion of active cortisol to cortisone and vice versa. In vitro, EDCs can interact with HSD11B1/2 followed by altered expression and activity in multiple target tissues of glucocorticoids [34]. For instance, TBT alters HSD11B2 expression and enzymatic activity in placental cells and thereby enhances cortisol deactivation [67].

Hormone synthesis and secretion

The steroidogenic enzyme cascade itself poses another potential target for EDCs. Alteration of steroid levels due to disrupted enzymatic activity affects the physiological role of steroids in vivo and in vitro.

A significant number of studies make use of the adrenocortical carcinoma-derived NCI-H295R cells as an established model for human steroidogenesis. H295R cells were treated with different EDCs measuring alterations in steroid levels and gene expression: enhanced secretion of effector hormones, such as aldosterone or testosterone were observed, whereas precursor hormones, such as 17-hydroxyprogesterone, pregnenolone, or 11-deoxycorticosterone, were decreased. Most pronounced effects were observed following 48 hours of exposure to TCDD, PCB 126/153, tributyltin (TBT), and 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) [48]. Importantly, effects of BDE-47-treatment on aldosterone and cortisol secretion suggest a time- and dose-dependent effect on steroidogenic pathways. Some EDC-driven effects appear to take at least 72 hours to overcome a "transcriptional and translational delay". The effects could be observed under stimulated and unstimulated conditions [59].

Additive effects of combinatory EDC treatment are hypothesised to be relevant for adverse outcomes in vivo, as realistic exposure is not limited to single compounds. Furthermore, combined toxicity has been suggested to aggravate effects of EDCs by synergistic or additive effects [48, 68]. Combination of EDCs, such as TCDD, PCBs, PFOS, TBT, and BDE-47, which had no effect in single use, was shown to exert relevant additive effects on steroidogenesis, for example, a decrease of DHEA [48]. Mixtures of chlorinated,

brominated, and perfluorinated substances were tested in different constellations in H295R cells for 48 hours. Most pronounced effects on glucocorticoids were found for mixtures of brominated and perfluorinated compounds – however, in levels 10 000 times higher than measured in human serum [68].

Most in vivo studies investigating endocrine disruption focus on effects on glucocorticoid levels. Suppression (e. g., in the presence of tributyltin, PFOS) [69–71] or elevation (e. g., in the presence of BDE-47, octyl-/nonylphenol, BPA) [36, 59, 72–75] of corticosterone levels was induced by a significant number of administered endocrine disruptors in animals. For instance, rats treated with PFOS (0.5 to 6.0 mg/kg body weight for 28 days) showed reduced corticosterone levels [70], whereas in utero BPA-exposed female rats had elevated basal corticosterone [76]. However, interspecies differences complicate a clear statement on the role of adrenal androgens, as rats lack CYP17 in adrenal tissue [32]. Certainly, systematic steroidobolomic studies at environmentally relevant concentrations and combined toxicity analyses are needed to ensure realistic conditions.

Hormone distribution

Secreted corticosteroids are distributed via the bloodstream and mainly bound to transport proteins. This ensures solubility and availability at target tissues, while metabolic degradation is attenuated. Glucocorticoids bind to corticosteroid-binding globulin (CBG), androgens and estrogens bind to sex hormone-binding globulin (SHBG), whereas for instance aldosterone, progesterone or DHEAS predominantly bind to serum albumin. Phthalates are able to bind to CBG and SHBG in silico with similar affinity as cortisol or dihydrotestosterone, the natural ligands [77, 78]. Hang et al. reported binding of several substances, e. g. phthalates, parabens, and benzophenones, to SHBG [79]. Enhanced serum CBG was detected in mice following oral PFOA exposure for 28 days [80]. Besides, different phthalates [81], BPA [82] or its substitute BPF [83] have been shown to bind to human serum albumin, additionally provoking confirmational changes of the protein structure, thereby potentially altering unbound fractions of steroids in the bloodstream.

Oxidative stress

Maintenance of redox balance due to extensive lipid metabolism and production of reactive oxygen species (ROS) during steroidogenic reactions appears to be an essential part of the adrenal metabolism. Exogenous disruptors, which promote ROS generation or impair antioxidant mechanisms, might imbalance a precise homeostasis. As cytochrome P450 enzymes conduct hydroxylation reactions and thereby produce free radicals, steroidogenesis is prone to ROS accumulation. Redox status in the adrenal gland is maintained via non-enzymatic, that is, via ascorbic acid (which shows highest concentration in the adrenal cortex), and enzymatic systems, for instance glutathione peroxidase or thioredoxin. Several EDCs, like bisphenols, phthalates, or parabens are known to contribute to ROS generation, and might additionally reduce the activity of antioxidant enzymes [37]. In an animal experiment, lowered activity of antioxidant enzymes and reduced glutathione (GSH) were detected following BPA (10 mg/kg body weight for 14 days) treatment in rats. Here, BPA-in-duced ROS provoked lipid peroxidation, quantified as malondialdehyde amount. Additionally, elevated corticosterone and ACTH levels were reported, as well as ZF hyperplasia. All observed effects were attenuated by concomitant treatment with antioxidant melatonin [36]. In H295R cell culture, superoxide gene and protein oxidation were induced in the presence of di-/monobutyl phthalate (DBP/MBP), while gene expression of essential CYP enzymes was decreased. Consequently, DBP treatment led to lowered androgen and corticosterone secretion [60].

Tissue homeostasis

The highly specific adrenal tissue organization allows the complex functions of the adrenal cortex in vivo. Therefore, disrupted cell differentiation and intercellular interaction might influence adrenal functionality.

Several histological studies report zone-specific hyperplasia of adrenocortical cells following EDC treatment [69, 71, 72, 75]. Treatment of adult rats with TBT resulted in hyperplasia of the pituitary and adrenal gland [71] with consecutive diffuse cellular hypertrophy, especially in ZG and ZF [69]. Similarly, lizards treated with octyl- and nonylphenol showed strong hypertrophy of steroidogenic cells and enhanced vascularization, concurrent with elevated ACTH which might have mediated adrenal growth [72]. Thickened ZF was detected in offspring of BPA-treated rats (40 µg/kg body weight/day), while reduced thickness and dysmorphic architecture of the ZR were detected [75]. Zone-specific hypo- or hyperplasia suggests distinct involvement of adrenocortical cell subpopulations in the toxic effects of EDCs.

Several in vivo studies report specific changes in adrenal weight, while other organs remain unaffected. For instance, relative adrenal weight was observed to be reduced at high doses of DBP (500–1000 mg/kg body weight/day for 14 days) and DEHP (750–1500 mg/kg body weight/day for 14 days) treated rats [84], whereas chronic exposure to BDE-47 (10–100 µg/kg body weight/day for 16 weeks) resulted in increased adrenal, but not in heart, liver or kidney weights [59].

Interestingly, effects on adrenal gland morphology seem to be sex-dependent. For instance, perinatally BPA-exposed female rats presented higher adrenal weights compared to their male littermates. While hyperplastic zona fasciculata could be detected in both sexes, the zona reticularis was specifically reduced or missing in male rats. Accordingly, hormone levels, receptor expression, and behavioural coping was found to be altered in a sex-dependent manner [75].

Histological studies revealed increased cytoplasmic accumulation of cholesterol and precursor hormones in the adrenal cortex [50, 69, 71]. Female rats treated with 100 ng TBT/kg body weight/day for 15 or 30 days resulted in an intense lipid accumulation [71]. Similarly, maternal exposure to DEHP (1–300 mg/kg body weight/day from GD14 until birth) resulted in a dose-dependent increase of lipid droplets in the adrenal glands of the offspring. Concurrently, LDLR and HMGCR expression was upregulated promoting increased lipid input and de novo cholesterol synthesis [50]. TBT might inhibit the transformation of cholesterol to steroids (early blockade of steroidogenesis), whereas DEHP might promote uptake and deposition of lipids in the adrenal cell.

In terms of tissue development, pathways involved in intercellular contacts, zone formation, proliferation and differentiation were shown to be inhibited by pre- and perinatal DDT-treatment (pregnant rats received 2–3 µg DDT/kg body weight/day). Developmental retardation in the ZR has been observed in the presence of insecticidal DDT: Immunohistochemical analyses showed reduced nuclear and elevated cytoplasmic fractions of β-catenin suggesting an insufficient activation of β-catenin/Wnt-signalling in the ZR [85]. The Wnt-pathway plays an important role in formation of tissue patterns, proliferation and differentiation [86]. Furthermore, Oct4 expression and Ki67-index were lowered in the ZR. Oct4 + cells form a cell pool involved in pluripotency and tissue homeostasis. Therefore, DDT surrogatively impairs the development of in utero exposed rats by downregulating essential pathways and consequently diminished cell proliferation and tissue repair [85]. Overall, the presence of DDT in the developing adrenal cortex resulted in dysmorphogenic alterations and retarded growth. Nonetheless, DDT is claimed for its adrenocorticolytic effects which led to the therapeutic use of its derivate mitotane in treatment of adrenocortical carcinoma (ACC) [87].

The chronic exposure to TBT caused loss of arcade patterns in the adrenal cortex and a random disposal of cells in male rats. Effects became more pronounced over time [69]. Similar histological lesions became apparent after DBP (100–1000 mg/kg body weight/day) or DEHP (250–1500 mg/kg body weight/day) exposure to male rats. A loss of adrenal architecture and degeneration of cells was described, as well as cellular congestion and loss of cell granularity [84].

Furthermore, local inflammation appears to be associated with chronic exposure to some endocrine disruptive agents. Several in vivo studies report immune cell infiltration in EDC-treated adrenal glands. TBT was shown to increase mononuclear and neutrophil cell counts in adrenal cortices of female rats alongside with an increased activity of macrophagous NAG (*N*-acetyl-β-d-glucosaminidase) and neutrophil MPO (myeloperoxidase). Besides, the number of mast cells was increased. Local inflammation concomitantly occurred in the pituitary gland [71]. Meanwhile, severe macrophage infiltration became evident after octyl- and nonylphenol treatment in adrenal tissue of lizards [72]. Furthermore, enhanced apoptotic markers, such as caspase-3 expression, suggest the induction of intrinsic apoptosis in the chronic presence of TBT. Thereby, elevated collagen deposition (fibrosis) in the adrenal cortex might be the consequence of replacing tissue defects induced by TBT [71]. Moreover, acellular immune regulation might be induced by the presence of DBP and DEHP in male rats. Serum levels of the proinflammatory IL-1 and TNFα were increased after 14 days of treatment. By alternating steroidogenic gene expression and reducing glucocorticoids levels, phthalates might exert cascadic effects on the adrenal gland and systemic immune balance as glucocorticoids can directly regulate a proinflammatory status [84].

Adequate vascular organisation in the adrenal cortex is required to maintain supply and transport of essential metabolites. Alkylphenols have been associated with enhanced vascularisation in adrenal tissue of lizards [72]. Meanwhile, dose-dependent angiectasis has been observed in the adrenal cortex of DBP/DEHP treated rats impairing efficient blood supply [84]. Reduced immunostaining of contractile and cytoskeletal elements of blood vessels was observed in adrenal glands of BPA-exposed rats. This likely results in reduced vascular contraction and congestion and disrupted integrity of adrenal vessels [36].

Disruption of endocrine circuits

Many in vivo studies investigating HPA-disruption report dissociated hormone levels of hypothalamic corticotropin releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and adrenal corticosterone: while BPA (10 mg/kg body weight/day for 14 days) enhanced glucocorticoid, ACTH, and CRH levels in the serum of treated rats [36], as well as their perinatally exposed offspring [73, 74], PFOS (0.5–6 mg/kg body weight/day for 28 days) suppressed levels of all three hormones [70]. Treatment with low-dose TBT (100 ng/kg body weight/day for 15, or 30 days, respectively), revealed elevated levels of corticosterone, while serum ACTH was suppressed [71].

The short-term exposure to ambient particulate matter ($PM_{2.5}$) as a ubiquitous environmental factor resulted in an activated HPA and therefore enhanced ACTH and glucocorticoids in an experimental [12], as well as in an epidemiological setting [88]. Particulate matter acts as an environmental transport medium of various chemical compounds, such as inorganic ions (nitric oxides, sulfur oxides), organic carbons, and heavy metals. Known endocrine disruptive substances may be included, for example, polycyclic aromatic hydrocarbons (PAH), or phthalates [89]. Exposed rats (average exposure of 62.6 µg/m^3) showed elevation of CRH, ACTH and cortisol levels, as well as an increased cytokine expression (IL-6, TNFα) in the hypothalamus, pituitary, and adrenal gland [12]. Accordingly, a randomized-controlled trial investigating effects of air purification detected enhanced levels of cortisol and cortisone associated with higher exposure to $PM_{2.5}$ (average personal exposure of 53.1 µg/m^3) [88].

To maintain glucocorticoid homeostasis in target tissues, hormone receptor levels appear to negatively correlate with steroid levels. Consequently, a downregulation of GR in the hypothalamus of female rats has been observed at elevated corticosterone levels in the presence of BPA (40 µg/kg body weight throughout pregnancy and lactation). This resulted in limited responsiveness to glucocorticoid feedback [75]. A 28-day treatment of rats with PFOS decreased levels of CRH, ACTH and corticosterone at all administered concentrations from 0.5–6 mg/kg body weight. Consequently, expression of CRH receptor (CRHR) and GR were dysregulated in the limbic system, hypothalamic PVN, pituitary, and adrenal gland [70]. Moreover, a mixture of phthalates, pesticides and BPA have been shown to induce hypomethylation and altered transcription of hippocampal HPA-related genes: MR and CRHR [76, 90], as well as hippocampal and hypothalamic FKBP1, an important regulator of glucocorticoid receptor expression in the brain [91].

Behavioural studies in rodents following exposure to EDCs have shown sex-dependent alterations of HPA physiology and associated behaviour [74, 75]. Pregnant rats orally administered with BPA 40 µg/kg body weight/day throughout pregnancy and lactation resulted in disruption of hormone secretion and hippocampal GR deficiency in the offspring. Behavioural testing, such as the forced swim test, revealed a less robust coping to stressors, suggesting anxiety and a depression-like phenotype [75]. Perinatally BPA-ex-

posed male rats (subcutaneous injections of 2 µg/kg body weight in dams) similarly showed depression-like behaviour. Contrary behaviour was seen in females, which presented which lower post-stress levels of corticosterone and ACTH and reduced anxiety-like behaviour in behavioural testing [74]. By targeting basal and stress-reactive HPA activity, EDC exposure might be associated with the development of stress-related disorders [75].

The RAAS addresses the adrenal ZG in systemic blood pressure control and salt balance. Disturbed feedback mechanisms in renin, angiotensin and aldosterone signalling might lead to hypertension or hypokalaemia. In vivo studies have found disrupted angiotensin II and renin expression in kidney tissues following maternal exposure to DEHP (0.25–6.25 mg/kg body weight) in rat offspring [92], suggesting the RAAS to be an additional target of EDC's action. Reduced systemic arterial blood pressure associated with reduced aldosterone levels was shown in the offspring of perinatally DEHP-exposed rats [93].

Discussion

Humans are exposed to a complex mixture of EDCs in low doses. These EDCs can accumulate in lipophilic tissues and tend to alter endocrine pathways and homeostasis. As discussed, the adrenal cortex represents a common toxicological target. Yet, the mechanisms of adrenocortical and HPA disruption and their involvement in adverse outcomes remain incompletely understood. Subsequently, challenges and gaps in EDC research targeting the adrenal system will be discussed.

A schematic overview of discussed potential targets for endocrine disruption in the adrenal cortex is shown in ▶ **Fig. 1**.

Dose

Levels of endocrine disruptive agents detected in serum or urine are usually in the nano- to low micromolar range, posing a chronic, but low-dosed exposure. Most studies indicated dose-dependent effects on steroidogenesis and further endpoints. However, most studies report adverse outcomes at unrealistic concentrations. The extrapolation of detected effects to realistic doses is certainly difficult to fulfil since EDC concentrations are rarely quantified in the adrenal tissue. Retrospective biomonitoring data are fundamental approaches to asses realistic EDC burden [25]. However, monitored exposure levels vary due to population, location, and occupation. For instance, reported serum BPA ranged from about 10 µM in exposed workers to nanomolar concentrations in the general population [63]. With respect to the adrenal cortex, accumulative and depositing effects are difficult to quantify and need to be considered in the future hazard identification of EDCs.

Time

The complexity of a chronic exposure to toxicants poses another intricacy in the study of adrenocortical disruption. Long-term studies are needed to detect pre- and postnatal effects of environmentally relevant concentrations of endocrine disruptors in vivo. Lifelong contact to endocrine disruptors raises the question how to transfer the model of a chronic exposure of human cells to laboratory assays. Furthermore, the time of exposure in life significantly contributes to the vulnerability to EDC-driven effects. Foetal development and differentiation, which are intimately regulated by hormones, might be impaired by an unfavourable environment in utero. Preliminary data suggest that in utero exposure may have effects on organogenesis and tissue organization of adrenal glands in animals [85, 86]. However, the implications on human development and pathogenesis are unclear.

Combinatory effects

Studies on combined toxicity of EDCs often lack a standardised approach. Sometimes equimolar concentrations are applied, whereas in other studies mixtures adjusted to environmental concentrations are used to screen for disruptive effects [48]. For a better understanding of EDCs' impact on the adrenocortical system, studies using doses equivalent to levels found in vivo are urgently needed.
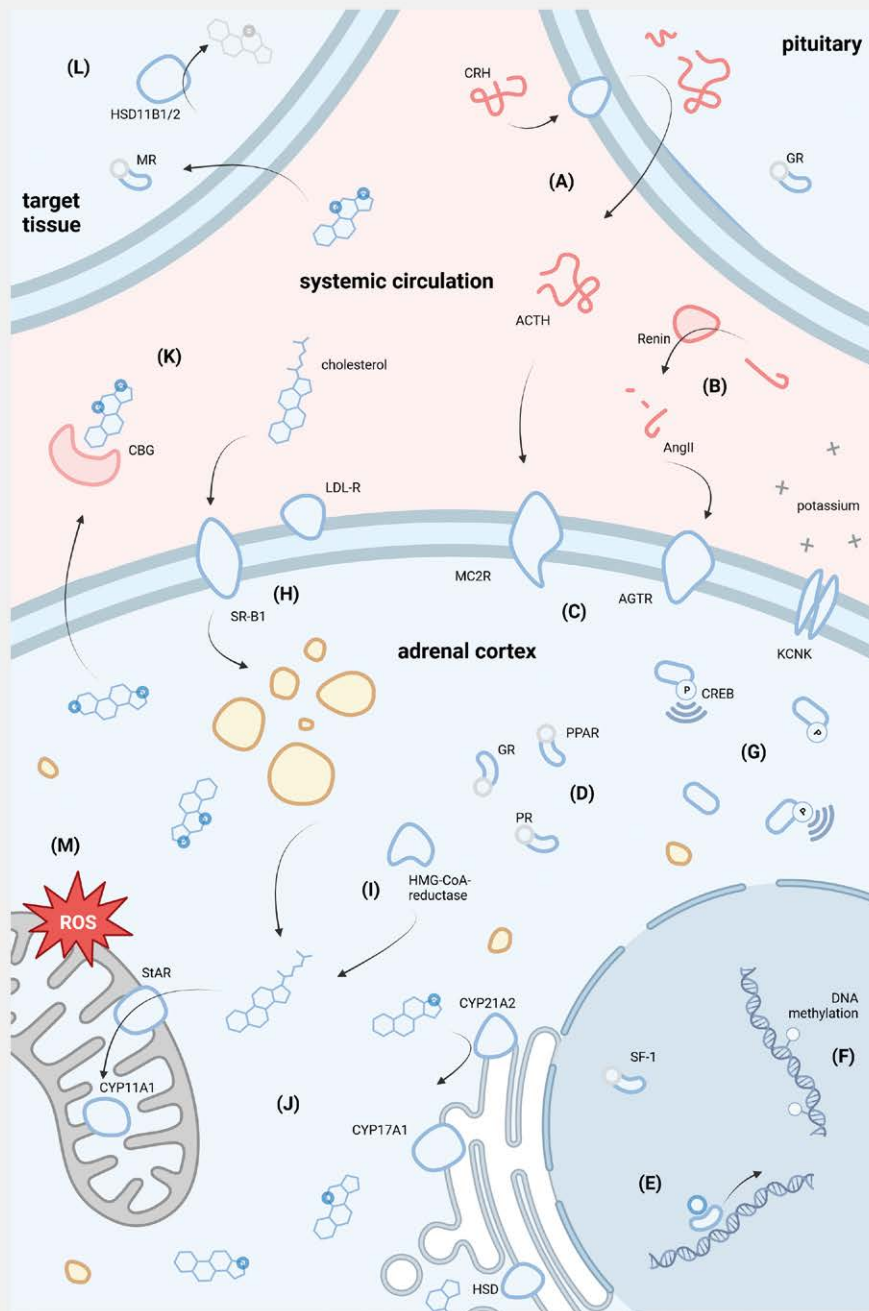
Common screening methods

Effects on steroidogenesis are often analysed using the NCI-H295R cell line, which has emerged as a screening tool for adrenocortical physiology and potential endocrine disruption [94–96]. This is already implemented in the screening panel for the hazard identification of newly developed chemicals, proposed by the OECD and the US-EPA. However, the established "steroidogenesis assay" is limited to an exposure of 48 hours and the detection of altered testosterone and estradiol levels in supernatant [97]. Clinically relevant gluco- and mineralocorticoids and the role of adrenal androgens, such as DHEAS have been ignored in many previous in vitro studies. Similarly, in vivo studies lack the detection of overall steroidogenesis disruption. A reliable, standardised, and straightforward approach detecting overall adrenal steroidogenesis is needed for thorough screening of endocrine disruption – desirably ahead authorisation of novel substances.

Circadian rhythmicity

Circadian steroid secretion has been rarely considered in environmental endocrinology. Secretion of glucocorticoids follows a circadian rhythmicity, which seems to be regulated by a finely tuned interplay of various mechanisms. Pulsatile secretion of hormones in hypothalamic nuclei and intrinsic oscillatory gene expression regulate the circadian rhythm in glucocorticoid secretion. Parallel endocrine and circadian disruption have been revealed in certain hypothalamic-pituitary axes, while at the same time clear mechanistic evidence of circadian disruption in adrenocortical cells and the HPA axis is lacking. How and if endocrine disruptors may influence diurnal glucocorticoid patterns has not yet been investigated [35], although relevant components of adrenal rhythmicity, for example, StAR [98], are known as targets of certain EDCs.

Epigenetics and DOHaD

Furthermore, epigenetic effects of EDCs via DNA methylation or histone modifications have been rarely considered in the adrenal cortex. However, by passing epigenetic alterations to progenies via trans- and

▶ **Fig. 1** Schematic overview of discussed potential targets for endocrine disruption in the adrenal cortex. EDCs can interfere with the adrenocortical system at several points. **a**: A superior system, such as the hypothalamus or pituitary gland, regulates glucocorticoid synthesis via the corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH). **b**: Similarly, aldosterone secretion is stimulated by the RAAS system by angiotensin II (AngII). **c**: External stimuli mediate their action via own receptors, such as the melanocortin 2 receptor (MC2R), the angiotensin II receptor (AGTR) or potassium channels (KCNK). **d**: The glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), peroxisome-proliferator-agonist receptor (PPAR) or steroidogenic factor 1 (SF-1) are essential for intracellular signalling of steroids on a transcriptional level (**e**). **f**: Via epigenetic modifications transcription of key genes is modulated. **g**: Signal proteins, such as CREB and their phosphorylation status mediate intracellular pathways. **h**: Cholesterol, as precursor of corticosteroids, is either uptaken by low-density lipoprotein receptor (LDL-R) or by scavenger receptor B1 (SRB1). **i**: De-novo synthesis is conducted by HMG-CoA-reductase. **j**: Corticosteroids are synthesized in the mitochondria and the smooth endoplasmatic reticulum mediated by steroid acute regulatory protein (StAR) cytochrome P450 enzymes (e. g., CYP21A2) and hydroxysteroid dehydrogenases (HSD). **k**: Steroids are bound to proteins, such as corticosterone binding protein (CBG). **l**: In their target tissues, steroids bind their nuclear receptor, activate hormone signalling pathways and are eventually inactivated. **m**: Steroidogenic reactions afford precise redox balance in the adrenocortical cell. Created with biorender.com. [rerif]

multigenerational inheritance, EDCs become a potential risk factor in developing organisms. Endocrine disruptors as a part of the developmental origins of health and diseases (DOHaD) reinforce our current responsibility for the health of future generations [24].

Adrenal-related disorders

Another central lack of knowledge is the potential clinical outcomes in the adrenal system induced or mediated by EDC exposure. The links between the exposome and steroid-related disorders are limited to association studies. However, EDCs were shown to alter levels and activity of the HPA axis, concomitant with central receptor expression, abundance, and distribution in the brain, affecting central responsiveness to glucocorticoids. Meanwhile, dysregulated HPA axis is observable in various disorders like depression, anxiety, metabolic dysfunction, obesity, and post-traumatic stress disorder. An inadequate reaction to altered environmental demands might result in a cascade of pathological events promoting pathogenetic processes [99]. Importantly, phases of pre- and early postnatal development mark the most vulnerable time window for an aberrant HPA programming by environmental factors [75]. Although enhanced levels of EDCs have been detected in patients with anxiety or depression [8], the mediation by an EDC-disrupted adrenal system in pathogenesis of stress-dependent mental disorders remains speculative.

Only a limited number of clinical trials investigated the association between the exposome and the prevalence of adrenal neoplasia in humans. While BPA serum levels were elevated in patients suffering from non-functional adrenocortical incidentaloma [25], PCB and organochloride concentrations in the adrenal cortex of patients with aldosterone-producing adenoma were significantly increased [26]. Both studies suffer from a small sample size and the unclear causality between individual exposure and disease incidence. However, 80% of EDCs are described as potentially tumorigenic promoting neoplasia in other steroidogenic tissue, such as ovaries, or testis. Among them, phthalates, heavy metals, and particulate matter were most often associated with endocrine neoplasia [100].

Meanwhile, polycystic ovary syndrome (PCOS), predominantly accompanied by hyperandrogenaemia, is a multifactorial disorder of the female reproduction tract. Epidemiological studies reported significantly elevated serum BPA levels PCOS patients, which correlated with enhanced androgen levels [101]. However, 20–30% of PCOS patients demonstrate an excess of adrenal precursor androgens, such as dehydroepiandrosterone (sulfate), potentially linking altered adrenal steroid production to reproductive disorders [102].

Conclusion

In this review, we have discussed the evidence on various targets and disruptive mechanisms of known EDCs within the adrenocortical system. Despite limitations, which we acknowledge, there is an increasing body of evidence that supports associations between the exposure to certain endocrine disruptive agents and adverse outcomes in the adrenal system. In conclusion, the adrenal cortex, its associated hormones, and their implication in the organism is indeed affected by the exposome. However, causal ascriptions of endocrine disruptive activity and the pathogenesis of steroid-related disorders, such as neurobehavioral disorders or endocrine neoplasia, remains unclear and requires further investigation. The abundance and extensive distribution of hazardous chemicals and developing evidence on their adverse health effects raises concern and emphasises the need to extend research of these EDCs.

Fundings

Conflict of Interest

The authors declare that they have no conflict of interest.

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