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The menace of endocrine disruptors on thyroid hormone physiology and their impact on intrauterine development

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Abstract The delivery of the appropriate thyroid hormones quantity to target tissues in euthyroidism is the result of unopposed synthesis, transport, metabolism, and excretion of these hormones. Thyroid hormones homeostasis depends on the maintenance of the circulating 'free' thyroid hormone reserves and on the development of a dynamic balance between the 'free' hormones reserves and those of the 'bound' hormones with the transport proteins. Disturbance of this hormone system, which is in constant interaction with other hormone systems, leads to an adaptational counter-response targeting to re-establish a new homeostatic equilibrium. An excessive disturbance is likely to result, however, in hypo- or hyper- thyroid clinical states. Endocrine disruptors are chemical substances forming part of 'natural' contaminating agents found in most ecosystems. There is abundant evidence that several key components of the thyroid hormones homeostasis are susceptible to the action of endocrine disruptors. These chemicals include some chlorinated organic compounds, polycyclic aromatic hydrocarbons, herbicides, and pharmaceutical agents. Intrauterine exposure to endocrine disruptors that either mimic or antagonize thyroid hormones can produce permanent developmental disorders in the structure and functioning of the brain, leading to behavioral changes. Steroid receptors are important determinants of the consequences of endocrine disruptors. Their interaction with thyroid hormones complicates the effect of endocrine disruptors. The aim of this review is to present the effect of endocrine disruptors on thyroid hormones physiology and their potential impact on intrauterine development.

Keywords Endocrine disruptors · T_4 · T_3 · TSH · Intrauterine exposure · Developmental disorders · Steroid receptors · TBG · TTR

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Introduction

According to the International Programme on Chemical Safety an endocrine disruptor is a chemical substance or mixture of substances that alters the function of the endocrine system(s) and consequently causes adverse health effects in an intact organism, or its progeny. Endocrine disruptors (xenobiotics) affect synthesis, secretion, transport, metabolism, and action of thyroid hormones (TH) at contaminant levels that are not lethal or carcinogenic, inducing toxic effects to wildlife and humans alike [1].

Approximately for the past 40 years, concerns have been raised about the potential for ecotoxicity due to exposure to Endocrine Disruptors. In "Silent Spring" (1962) Rachel Carson presented examples of impaired development in birds, mammalian gonadal dysfunction, and tumorigenesis in humans. In the meantime, much progress has been made on hazard characterization, dose–response assessment, and exposure assessment of endocrine disrupting chemicals. Increasing evidence suggests that, in addition to classical routes of contaminant exposure (direct exposure to contaminants or consumption of contaminated food and water), maternal exposure may be of great significance when studying either viviparous or oviparous organisms. An additional exposure of maternal

origin in mammals is breast milk contamination [2, 3]. Psychotic drugs have been found in measurable amounts in plasma and tissues (e.g., brain) of the nursing infant, suggesting transmammary secretion after maternal ingestion [4]. In addition, mechanisms of action as well as the potential adverse effects elicited by "antihormones" have been studied. Colborn et al. pointed out that humans are unique among species to modify the environment rapidly through technology, either negatively or positively [5]. In many cases the intentional release of compounds through agricultural, industrial, and municipal activities has influenced the health of wildlife and humans via alteration of survival, biodiversity, and genetic variety. Increasing evidence suggests that many endocrine-disrupting compounds may interfere with the function of endogenous endocrine systems by interupting several points along the classic endocrine axes. Dichloro-diphenyl-trichloroethane (DDT) and its metabolites, polychlorinated biphenyls (PCBs), and dioxins can interact with sex steroids, corticoids, and TH in several vertebrate classes. Through this interaction with the endocrine axes, they potentially modify developmental and reproductive physiology. Reported abnormalities in laboratory animals and wildlife exposed to endocrine disruptors include feminization of males, abnormal sexual behavior, birth defects, altered sex ratios, decreased sperm density, testicular cancer, reproductive failure, and thyroid dysfunction. The aim of this review is to present the effect of endocrine disruptors on the regulatory action of TH and their potential impact on intrauterine development.

Physiology of the hypothalamic–pituitary–thyroid axis

Homeostasis

A functioning thyroid is evident in animal species as primitive as the lamprey. Thyroid gland secretes thyroxine (T_4), triiodothyronine (T_3), and small amounts of mono- and diiodotyrosines (MIT and DIT). In most species, T_4 is present at higher concentrations in blood than T_3, and it is the primary effector in the negative feedback regulation of the hypothalamic–pituitary–thyroid (HPT) axis. The pituitary thyroid-stimulating hormone (TSH) coordinates thyroid gland activity in most species with the exception of some species of cyclostomes (lamprey and hagfish) [6].

The follicular organization of the thyroid gland, with the thyroid cells forming an epithelial layer surrounding a fluid-filled lumen called "colloid", has been conserved in all vertebrate classes. Hyperplasia of thyroid follicular cells is induced by excessive TSH. These cells synthesize an iodinated glycoprotein called thyroglobulin. Iodine, an essential element for TH synthesis, is accumulated in the form of iodide or iodate ion, from the diet by terrestrial verterbrates, and from the diet and seawater by amphibians and fish. The iodate ion is converted to iodide in the stomach. Iodide is easily absorbed by the gastrointestinal tract and then distributed in extracellular fluid [7]. The Na^+/I^- symporter is an integral plasma membrane glycoprotein that mediates active iodide transport across the basement membrane of the thyroid follicular cells [8]. At the apical border, a protein, called pendrin, transporter of chloride and iodide, moves iodide into the colloid, where its organification takes place via MIT and DIT residue formation and condensation to T_4 and T_3. Thyroid hormones secretion is a TSH-stimulated cAMP-coupled process.

Thyroid hormones are transported in serum bound to carrier proteins. There are considerable species differences in the synthesis and affinity of transport proteins. In humans and in large eytherians, TH are bound reversibly to three transport proteins: thyronine-binding globulin (TBG), transthyretin (TTR)—formerly called thyroxine-binding prealbumin (TBPA)—and albumin. Transthyretin is responsible for 10–15% of the TH binding activity in human plasma [9], while it is the major TH transport protein in the bloodstream of birds, herbivorous marsupials, and small eutherians [10]. It is the only TH-transport protein synthesized in the cells of the blood–cerebrospinal fluid barrier, in addition to its synthesis in the liver. In rats, about 12% of total proteins synthesized and about 50% of proteins secreted by the choroid plexus is TTR [11]. Access of albumin and TBG from the bloodstream to the brain and the cerebrospinal fluid is limited. Transthyretin appears to play a major transporting role in the extracellular space of the mammalian, avian, and reptilian brain [12]. Transthyretins of non-mammalian vertebrates, such as birds, amphibians, and fish, show higher affinity for T_3 than for T_4 [12, 13]. Moreover, the presence of all three TH-transport proteins (TBG, TTR, and albumin) was identified in human trophoblast, suggesting that human placenta may intervene in materno-fetal TH transport [14]. 0.04% T_4 and 0.4% T_3 are unbound. These 'free' fractions are responsible for hormonal activity. In many in vitro studies, it has been shown that TH-transport proteins have a permissive effect on efflux of TH, probably by facilitating diffusion of these hormones through the water layer around the cell [15].

The daily secretion of the normal human thyroid gland is about 100 nmol T_4, about 5 nmol T_3, and less than 5 nmol of metabolically inactive reverse T_3 (rT_3). Most of the T_3 present in the serum of the vertebrate species is probably derived by removal of one iodide from the outer tyrosine ring (5′ deiodination) of T_4. Three monodeiodinase isoenzymes, involved in catabolism of the

'prehormone' T_4, have been identified and cloned (D1, D2, and D3, respectively). Both D1 and D2 enzymes generate the active hormone T_3 by reductive deiodination of the phenolic (outer) ring of T_4. Deiodinase 1 is the major isoform in liver, kidney, thyroid, and pituitary and it is distributed widely among extrathyroidal human and rat tissues. There are species-specific differences in D2 expression [16]. It is noteworthy that, the D2 activity is detectable both in human and rat heart [17]. The thyroid gland is the major source of circulating T_3 in the rat. In humans the majority (>80%) of the circulating T_3 is derived from the enzymatic deiodination of T_4 to T_3 in peripheral tissues. Hepatic D1 is assumed to produce most of the T_3 found in circulation, while thyroid D1 also seems to contribute to circulating T_3 [18]. Inactivation of T_4 and its product T_3 occurs by deiodination of iodothyronines at the tyrosyl (inner) ring. This reaction is catalyzed both by D3 and D1, which has broader-substrate specificity.

Thyroid hormones action at cellular level

The effects of T_3 at the genomic level are mediated by the nuclear thyroid receptor (TR). The four TR isoforms are protooncogene products derived by differential splicing of two different genes (TRα and TRβ). In chicken and certain amphibians (e.g., *Xenopus laevis*) shorter TR transcripts have been identified [19]. Thyroid receptor acts on TH response elements (TREs) in both the absence and presence of its ligand (T_3). The effect of TR in the absence of T_3 is referred to as ligand-independent activity [20]. Not all TR isoforms can bind their ligand (i.e., the TRα2 isoform lacks the ability to bind ligand due to loss of 40 amino acids in the COOH-terminal hormone binding domain) [21]. Differential interaction with other proteins, including members of the steroid receptor superfamily, may play a role in TH physiology. By virtue of sequence homology, TRs are members of a large superfamily of nuclear hormone receptors including the steroid, vitamin D, and retinoic acid receptors that share a similar DNA-binding domain. TR and estrogen receptor (ER) bind to parts of the same steroid response elements (SRE) of target genes and can compete with each other [22]. Under this hypothesis, the result of TR and ER interactions would depend on the presence of ligands and the affinity for SREs. Indeed, this was first demonstrated for the vitellogenin estrogen response element (ERE) by Glass et al. (Fig. 1) [23]. Binding of TR to the ERE decreases ERα-mediated transactivation. Although the association between TR and ER is not entirely clear, the likelihood of a relationship is strong and complicates endocrine disruptor interactions with either receptor.

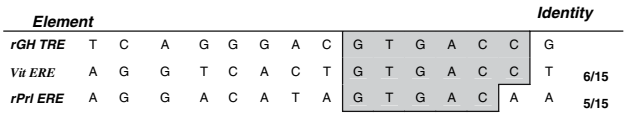

Fig. 1 Comparison of rGHT_3 response element (rGH TRE) to other steroid hormone response elements. Direct sequence alignment of the rGH TRE with the Vitellogenin A2 estrogen response element (Vit ERE), and the rat prolactin estrogen response element (rPrl ERE) [23]

Thyroid hormones and other endocrine axes

Thyroid hormones affect protein and lipid metabolism in fish (heteropneustes fossils) as in higher vertebrates, e.g., reptiles (alligator missipiensis) and rodents [24–28]. In mammals and birds, TH are also involved in regulation of temperature and stimulation of thermogenesis [29]. Thyroid hormones accomplish similar functions in some reptiles where they induce basking behavior [30]. Thyroid hormones are also necessary for growth. They stimulate growth hormone (GH) release from the pituitary and are necessary for bone elongation and maturation [31, 32]. In amphibians, TH regulate several genes involved in tail resorption, limb development, and gut regression [33–35]. A positive feedback mechanism (called "spiraling") may occur in amphibians. Some hypothalamic–pituitary stimulation leads to increased TH synthesis, which, in turn, induces maturation of the median eminence, leading to increased TSH and, subsequently, increased TH secretion [24]. Rapid developmental changes analogous to amphibian metamorphosis occur in all vertebrate classes. Thyroid hormones appear to be involved in these events [24]. In fish, TH enhance metabolism, somatic growth and the metamorphosis that follows hatching [36]. In the flatfish, the regulation of eye, mouth, and neural structure metamorphosis is associated with the peak concentration of T_4 [32]. In the salmon, the developmental changes occur before migration from fresh water to salt water (smoltification). After smoltification, serum T_4 concentrations decline, suggesting that the regulation of osmotic pressure is also influenced by TH. Thyroid hormones are also necessary during mammalian fetal development, playing an active part in brain development and auditory function [37–39] (Fig. 2).

Thyroid hormones and cortisol actions in different species are closely interrelated. In fish, TH play at least a supportive role in seawater acclimation and may interact with both the cortisol and GH/insulin-like growth factor 1 (IGF-1) axes. T_3 treatment upregulates the number of gill cortisol receptors in rainbow trout and increases the in vitro capacity of cortisol to increase gill Na^+, K^+ATPase activity [40]. In the Atlantic salmon, T_3 increases the number of gill cortisol receptors, and this effect is potentiated when T_3 is administered with GH [41]. T_4 treatment

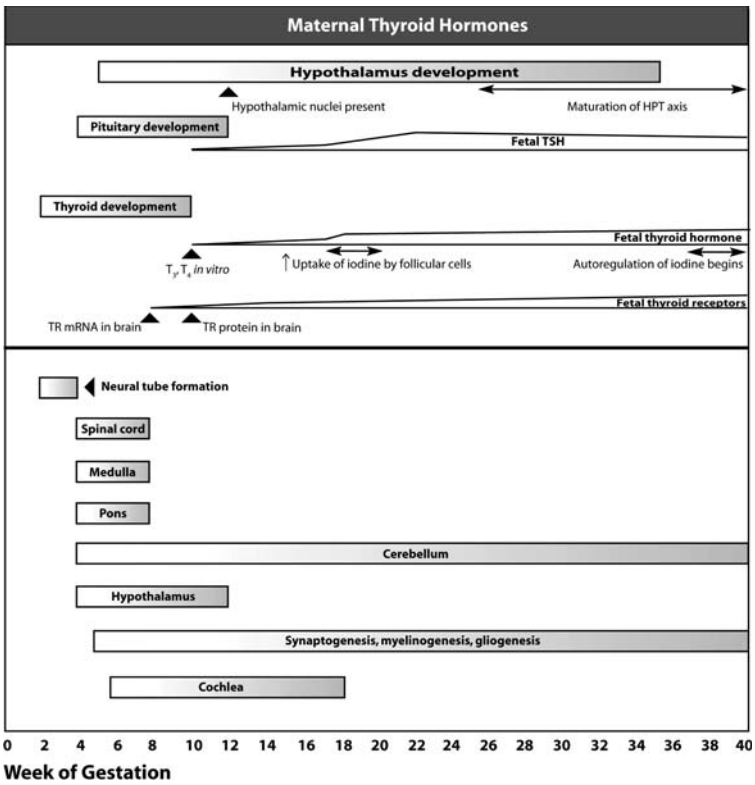

Fig. 2 Fetal development of the HPT axis components during gestation with regard to the development of major TH-dependent parts of the nervous system

in thyroidectomized rats increased basal plasma corticosterone and corticosterone binding globulin (CBG) concentrations during either short- or long- term hyperthyroid state [42]. The available data suggest that hyperthyroxinemia is associated with hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis, although the level of the axis that is primarily affected is unclear. TR interaction with the ERE has been suggested [22, 23]. Moreover, TH regulate the synthesis and secretion of several hormones, including GH and IGF-1 [40]. Not surprisingly, TH are necessary for a galactopoietic response to GH and prolactin (PRL) in mice [43]. A possible explanation is that the expression of ER, PRL, and progesterone receptors in mammary gland and liver of female rats during pregnancy and early postpartum is regulated by TH [44]. In addition, T_3 can stimulate the transcription of GH mRNA and GH synthesis in rat pituitary tumor cells [45]. Absence of GH secretion has been observed in the pituitaries of hypothyroid rats [46]. The 5′-flanking region of the rat GH gene contains a TRE between position –167 and –190 that binds TR in vitro [47, 48]. Vitamin D inhibits the transcriptional response of the rat GH gene to T_3 by interference of the vitamin D receptor on this TRE [48].

Endocrine disruptors and thyroid hormones

Several chemicals act as thyroid ‘antihormones’ through disruption of TH bioavailability. The role of endocrine disruptors on different points of TH physiology is analyzed below (see also Table 1).

TSH stimulation and iodination inhibition

Iodide organification is a TSH-stimulated process. It has been postulated that some agents may act via modification of TSH action. Theophylline may potentiate TSH action through its inhibitory effect on phosphodiesterase, leading to intracellular increase of cAMP concentration. In the low-iodine fed rat, the presence of pituitary is required for the enhancement of the goitrogenic effect by methylxanthines [97]. PCB 95 was found to exert direct and potent actions on the type 2 and 3 ryanodine-sensitive Ca^{2+} release channels, which are expressed and hormonally regulated in rat pituitary, inducing a direct TSH release from the pituitary [98].

It is well known that certain large anions such as thiocyanate and perchlorate are competitive inhibitors of iodide accumulation in the thyroid [99–101]. Perchlorate and thiocyanate anions resemble iodide in size and charge, and they may interact with the same site on the Na^+/I^- symporter molecule. Thiocyanate anion is not concentrated in the thyroid glands of most species studied, being rapidly metabolized after translocation into the thyroid follicular cells [99]. Perchlorate anion is 10–100 times more potent than thiocyanate as an inhibitor of iodide accumulation in a variety of in vivo and in vitro systems. Perchlorate salts are found in rocket fuel, fireworks, and fertilizers [100].

Table 1 Substances that act as Endocrine Disruptors of the HPT axis in different animal species and humans (adapted from Ref. 49)

Categories of substances	Substance	Study	Species	Route of exposure	Dose	Effect	References
Benzamidazoles	Carbendanzin	In vivo	Beaver	Food	1,000 mg/kg	Increase in the weight of the thyroid gland	Til et al. [49]
		In vivo	Beaver	Food	2,000–5,000 mg/kg	Increase in weight of the thyroid gland	Reuzel et al. [50]
		In vivo	Wistar rat	Food	10,000 mg/kg	Increase in the rate of diffuse replication of the C cells of the thyroid gland	Til et al. [51]
Chlorinated cyclodienes and camphenes	Chlordane	In vivo	Rat	Food	121–203 mg/kg	An increase in the incidence of follicular neoplasms	NCI [52]
	Mirex	In vivo	Rat	Food	0.7 mg/kg × day	Demonstration of cystic masses and histological alterations at the thyroid gland	IRIS [53]
		In vivo	Rat		50 mg/kg	Ultrasonographic changes at the thyroid follicular cells	Singh et al. [54]
	Campheclor	In vivo	Rat		540 ppm	An increased incidence of thyroid gland tumors	
DDT, derivatives and metabolites	DDT = Clofenotane	Observation			6 mg/kg × day	Hypothyroidism	Jefferies et al. [55]
Dithiocarbamates	Maneb	In vivo	Dog	Food	400 ppm	Hyperplasia of follicular cells of the thyroid gland	
		In vivo	Monkey	Food	300 ppm	Increase in weight of the thyroid gland	
		In vivo	Mouse	Food	240 ppm	Decreased levels of thyroid hormones	
		In vivo	Wistar Rat		20 mg/kg	A decrease in the levels of TSH, T_3 and T_4.	Laisi et al. [56]
	Thiram	In vitro	Hamster		10 μM	Effect on the activity of hyperoxidase or disorders in the iodization of thyroglobulin	Marinovic et al. [57]
		In vivo	Dog	Food	1,000 mIU/ml	Hyperplasia of follicular cells of the thyroid gland	
	Ziram	In vivo	Rat	Food	0.7 mg/kg × day	Histological changes of the thyroid gland	Keizo et al. [58]
	Ziram	In vitro	Hamster		5 μM	Inhibition of the activity of hyperoxidase	Marinovic et al. [57]
Hexachlorocyclohexane and Isomers	Gamma-HCH = Lindane	In vivo	Heteropneustes fossilis		8 ppm	Decreased levels of thyroid hormones	Yadav and Singh [59]
Organo-phosphorpesticides	Dimethoate	In vivo	Chicken			Decreased levels of thyroxine	Maiti [60]
		In vivo	Mouse			Decreased levels of T_3 and T_4	Maiti and Kar [61]
	Malathion	In vivo	Rat	Oral route	0.06 mg/animal	A significant suppression of T_3 and T_4	Akhtar et al. [62]
	Methylparathion	In vivo	Channa punctatus		52 μg/l	Increase in T_3 concurrent increase in TSH	Ghosh et al. [63]

Table 1 continued

Categories of substances	Substance	Study	Species	Route of exposure	Dose	Effect	References
Triazines-triazoles	Amitrol = Aminotriazol	In vivo	Chicken	Transperitoneally	500 mg/kg/day	Increase in the ratio of the weight of the thyroid gland to the body weight	Wishe et al. [64]
	Atrazine	In vivo	Salmon		6.5 μg/l	Promotes the production of thyroid hormones	Waring et al. [65]
		In vivo	Mouse	Food	1,000–2,192 mg/kg	Thyroid gland tumors	Innes et al. [66]
		In vivo	Rat	Transperitoneally	5 mg/kg	Inhibition of iodine uptake from the thyroid gland	Alexander [67]
		In vivo	Rat		5 mg/kg × day	An increased incidence of thyroid gland tumors	WHO [68]
	Triadimefon	In vivo	Rodents			An increased incidence of thyroid gland tumors	Hurley [69]
Pesticides	Acetoclor	In vivo	Rodents			Tumors, decrease in the levels of thyroid hormones, increase in TSH and clearance of T_4	Hurley [69]
	Alaclor	In vivo	Long Evans rat	Food	126 mg/kg × day	Effect in the level of thyroid hormones	Wilson et al. [70]
	Heptaclor	In vivo	Malealbino rat	Oral route	0.5 mg/animal	A significant suppression of T_3 and T_4 concurrent increase in TSH	Akhtar et al. [62]
	Nitrofen	In vivo	Mouse			A decrease in the levels of T_4.	Gray and Kavlock [71]
	Propanil	In vivo	Rodents			An increased incidence of thyroid gland tumors, a decrease in the levels of T_4.	Hurley [69]
Chlorophenoles and benzens	2,4 dichloro- phenols	In vitro				An increased binding with transthyretin	Van der Berg et al. [72]
	Hexachloro-benzene (HCB)	In vivo	Hamster	Food	200 mg/kg food	An increased incidence of thyroid gland adenomas	Cabral [73]
		In vivo	Rat	Food	9.5 mg/kg × day	A decrease in the levels of total and free T_3, T_4	Besten [74]
Phthalates	Di-(2-ethylexyl)-phthalate(DEHP)	In vivo	Mouse	Food		An decreased incidence of thyroid gland tumors	N.T.P. [75]
		In vivo	Rat		1,000 mg/kg × day	Decreased levels of thyroxine	ATSDR [76]
	Di-*n*-butyl-phthalate(DBP)	In vivo	Rat	Oral route	750 mg/kg × day	Anomalous tumor or microscopic changes at the thyroid gland	Smith [77]

Table 1 continued

Categories of substances	Substance	Study	Species	Route of exposure	Dose	Effect	References
Polychlorinated biphenys and biphenyl ethers	PCB	Observation	Seal	Food	1.5 mg/day	Extremely low levels of retinol, total and FT_4 and total T_3	Brouwer et al. [78]
		Epidemiological	Human			Disorders of thyroid function	Safe [79]
	PCB 153	In vivo	Rat		16 mg/kg/day	Decreased levels of T_4 at weaning	Ness et al. [80]
	PCB 77	In vivo	Rat	Perinatally	8 μg/kg/day	Decreased levels of thyroxine at the descendants	Seo et al. [81]
	PCB Aroclor 1254	In vivo	Rat		0.1 mg/kg/day	Decreased levels of thyroxine	Gray et al. [82]
		In vivo	Rat		1 mg/kg	Decreased levels of thyroxine at the descendants	Goldey et al. [83]
Brominated & polybrominated biphenyl ethers (PBDE)	decaBDE	In vivo	Mouse	Food		Hyperplasia of follicular cells of the thyroid gland	EHC 162 [84]
		In vivo	Rat	Food	80 mg/kg	Hyperplasia of the thyroid gland	EHC 162 [84]
	octaBDE	In vivo	Rat	Food	1,000 mg/kg	Hyperplasia of the thyroid gland	Great Lakes Chemical Corporation [85]
	PBBs = Brominated Biphenyls	Epidemiological	Human	Work		Hypothyroidism	Bahn et al. [86]
Dioxins	2,3,7,8-TCDD	In vivo	Rat	Perinatally	0.1 μg/kg/day	Decreased levels of thyroxine at the descendants	Seo et al. [81]
Metals	Lead	Epidemiological	Human		>440 μg/l	Decreased levels of thyroxine	Robins et al. [87]
	Aluminum	In vivo	Fish			Increase in T_4	Waring and Moore [88]
	Tributyltin oxide = bis(tributiltin)oxide	In vivo	Rat	Oral route	100 mgTBTO/kg	Alterations in the level of thyroid hormones at the thyroid gland and the pituitary gland	Funahashi et al. [89]
		In vivo	Rat	Food	80 mgTBTO/kg	Decreased levels of T_4, TSH, increase in LH, changes at the thyroid gland and the pituitary gland	Kranjnc et al. [90]
		In vivo	Rat	Food	2.5 mg/kg	Decrease in the weight of the thyroid gland	Wester et al. [91]
Other substances	Carbon disulphide	Epidemiological	Human			Decreased levels of T_4	Cavalleri [92]
	Phenol	In vivo	Rat	Water	2,500 mg/l	An increased incidence of carcinomas from C-cells	NCI [93]
	Resorcinol	In vivo	Rat			Goitrogenic	Doniach and Fraser [94]
		Epidemiological	Rat			Goitrogenic	Lindsay and Gaitan [95]
	Vinyl acetate	In vivo	Rat			Thyroid gland cancer	Lijinsky and Reuber [96]

The second major group of goitrogens inhibits the formation of iodinated tyrosines and the peroxidase-catalyzed coupling of tyrosine residues in the thyroglobulin, suggesting that the site of action in the two processes is similar. Some of these goitrogens might act via competitive inhibition of TPO [102]. Endocrine disruptors known to act in this way are: (a) thionamide compounds (e.g., 6-*n*-propylthiouracil, thiourea, 1-methyl 2-mercaptoimidazole, *p*-aminobenzoic acid, sulfonamides); (b) aminoheterocyclic compounds (e.g., *p*-amino salicylic acid, 1-butyl-3 *p*-tolylsulfonyl urea); and (c) substituted phenols (e.g., resorcinol, salycimide) [6]. Thiocarbamide drugs—particularly propylthiouracil, methimazole, and carbimazole (group a)—are potent inhibitors of thyroid peroxidase (TPO) and block thyroid hormone synthesis. In addition thiocarbamides inhibit deiodination by D1 deiodinase. Many minerals alter the synthesis of TH mainly through interference with iodide concentration and its binding by the thyroid gland. Calcium, nitrate, bromine, rubidium, and fluorine are strongly goitrogenic. Lithium carbonate, employed for treatment of affective disorders, can produce goiter in susceptible persons [97].

Flavonoids, a class of naturally occurring plant derivatives with estrogen-like activity, inhibit TPO and ATPases. Antithyroid effects of potent flavonoids, such as fisetin, kaempferol, naringenin, and quercetin, have been observed in humans and experimental animals [103, 104]. In postmenopausal women, dietary intake of the soy isoflavones (particularly genistein) is recommended for the relief of menopausal symptoms [104]. Fourteen trials assessing the effects of soy on at least one measure of thyroid function in healthy, euthyroid, iodine-replete adult subjects provided little evidence that soy foods or isoflavones affect adversely thyroid function [105]. However, low concentrations of the isoflavone genistein have been shown to displace completely the TTR-bound T_4 in human serum and cerebrospinal fluid (CSF), indicating a potent competition between soya isoflavones and T_4 for binding to TTR [106]. It is suggested that isoflavones might alter the balance between "free" and "bound" TH fraction with possible effects on the feedback regulation of HPT axis.

An experimental study describing the effects of long-term treatment with the phytoestrogen resveratrol (RES) in ovariectomized rats demonstrated that RES induced an increase in total serum T_3 levels and enhanced TSH beta mRNA in adenohypophysis of the rodents indicating a state of relative hypothyroidism [107]. Male Long-Evans rats exposed (from conception to time of testing or tissue collection) to a diet rich in isoflavones showed increased total T_3 levels [108]. Studies have shown that exposure of infants to soy-based formulas complicates management of congenital hypothyroidism [109–112]. Of note, estrogen replacement therapy in postmenopausal women led to higher mean TSH levels as compared to control untreated postmenopausal women [113] while in humans, isoflavones such as genistein or daidzein, can exceed the potency of estradiol, especially in infants fed soy-based formulas as a sole source of nutrition [114]. In addition, in hypothyroid postmenopausal women treated with thyroxine, estrogen replacement therapy increases the need for thyroxine [115]. The mechanism suggested is that oral estrogen therapy, raises the circulating levels of TBG, thereby increasing the bound fraction and decreasing the "free" (bioactive) fraction of circulating T_4 [116]. It seems that both estrogens and phytoestrogens have been shown to increase circulating TBG. Exposure of humans to isoflavones or estrogen-like disruptors might alter, at least in infants circulating "free" TH levels influencing, thus, the HPT axis homeostasis. More clinical and epidemiological studies are needed to assess the extent of influence of these natural and synthetic endocrine modulators on the HPT axis based on the mode of action as well as dose–response relationships.

Besides decreasing TPO activity, genistein causes structural changes of this enzyme in rats by covalent binding leading to a new antigenic form of TPO (a neoantigen) that could induce recognition by the immune system. This action of genistein in rats suggests that soy consumption could be involved in the development of autoimmune thyroid disease [104].

Endocrine disruptors and transport proteins

Transport proteins

Changes in the plasma levels of TH transport proteins or competition on their binding sites by endocrine disruptors can alter the dynamic equilibrium between 'bound' and 'free' plasma TH.

Androgenic steroids and glucocorticoids lower TBG, as does major systemic illness as well. Several drugs such as salicylates, phenytoin, phenylbutazone, and diazepam may bind to TBG, displacing T_4 and T_3, producing in effect a low-TBG state. Furosemide is highly reactive with TBG. Similarly, drugs known to interfere with T_4 binding to mammalian TTR (higher affinity for T_4 than for T_3) are: penicillin, salicylate, salsalate; a noniodinated cardiac inotropic agent, milrinone; a synthetic plant flavonoid EMD 21388; and the anthranilic acid class, such as flufenamic, meclofenamic, and mefenamic acids [117, 118]. Interestingly, drug interactions with TTR may be important when this protein becomes a major circulating T_4-transport protein, as it occurs in patients with complete or partial TBG deficiency, or when serum T_4 is markedly elevated. Such interactions may also be important where TTR is the dominant tissue T_4-transport protein, as it is the case in the

choroid plexus [118]. It seems that in humans only under certain circumstances described above and when high doses are administered disturbances of TH homeostasis could result.

This mode of action has also been demonstrated for the hydroxylated PCBs, dibenzo-p-dioxins, and dibenzofurans by numerous studies [119–123]. Although information concerning the effect of binding of thyroid-disruptors to transport proteins on HPT axis homeostasis is scarce, the following data are cited. In an in vitro competitive binding assay none of the tested hydroxylated PCBs, PCDDs, and PCDFs inhibited T_4 binding to TBG. Some T_4 derived compounds, such as tri-iodophenol were tested on both TTR and TBG. Tri-iodophenol inhibited T_4-TTR binding but not T_4 binding to TBG [124]. Nevertheless, an experimental in vitro study indicated that amphibian TTRs have the ability to bind diethylstilbestrol (DES) at a dose of 8 nM with similar affinity to T_3, raising the possibility that binding of DES to TTR might induce the temporary elevation of plasma free T_3 levels followed by acceleration of cellular T_3 uptake [117]. Also, in an in vitro study it has been shown that binding of DES and pentachlorophenol to chicken and bullfrog TTR may weaken their effects on target cells by lowering their plasma "free" concentrations. This may eventually affect TH homeostasis in vivo by altering the plasma "free" TH levels [121]. Few comparable studies have been made in wildlife (nonhuman, nonrodent) species. Brouwer at al. demonstrated that, the carrying capacity for T_4 in the common seal is influenced by the dietary levels of PCBs [6, 125]. In studies of wild and captive birds, a reduction in plasma total T_4 levels, sometimes associated with thyroid enlargement, has been reported in gulls, pigeons, and guillemots fed diets contaminated with PCBs, *p,p′*-DDE, or dieldrin [6]. Two studies examined the effect of organochlorine contaminants on thyroid function in fish [126, 127]. These studies reported a lowering of plasma T_4 and T_3 levels in the coho salmon (*Oncorhyncus kisutch*) and rainbow trout (*Oncorhyncus mykiss*) fed PCB- and Mirex-contaminated diets. However, there was no evidence of endocrine disruptor-induced thyroid enlargement in either species. A possible explanation is that in salmonid fish, as opposed to mammals and birds, plasma TH bind prealbumin [126].

Brouwer et al. also investigated the interactions of persistent environmental organohalogens with the mammalian thyroid system. They demonstrated: (a) competition between these compounds and TH (high affinity) for binding to TTR; (b) induction of the hepatic enzyme UDP glucuronyl transferase (UDPGT) activity that catalyzes glucuronidation of the increased "free" T_4 fraction to eliminate it; and (c) inhibition of hepatic D1 that catalyzes the conversion of T_4 to T_3 [122]. Structure–activity relationship studies have shown that PCBs with *ortho*-substitution and hydroxylated PCB metabolites demonstrate significant affinity for binding to TTR (Fig. 3), consistent with measured biological activities of these PCBs, including effects on cell dopamine content, Ca^{2+} homeostasis, and protein kinase C translocation in neuronal cells and brain homogenate preparations [98, 128]. When female Sprague–Dawley rats were exposed to *ortho*-PCB congeners 95 or 101 T_4 levels decreased, while T_3 or TSH levels remained unchanged suggesting interruption of the feedback mechanism(s) of the HPT axis [98]. Exposure to PCB 95 or PCB 101 decreased thyroid colloid area in these rats. Thus, the authors concluded that the HPT axis appears to be a target of *ortho*-substituted PCBs. In general, PCB mixtures and related compounds cause prolonged reductions in circulating T_4 levels, but there may or may not be a compensatory increase in plasma TSH levels in adult and neonate rats [129–131]. It is possible that the nonplanar, i.e., *ortho*-substituted PCB congeners may bind TH

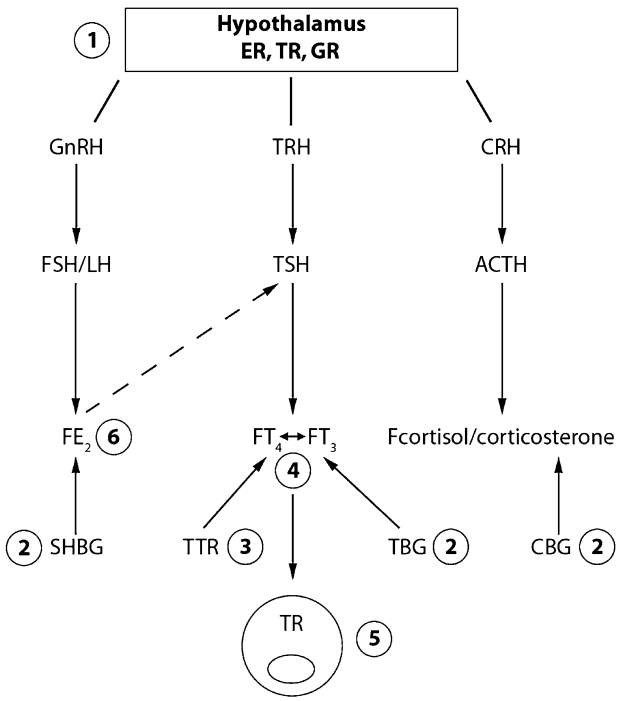

Fig. 3 Sites of action of endocrine disruptors on HPT axis in different animal species. Endocrine disruptors: (1) bind to ER, TR and GR at the level of *hypothalamus*, (2) increase the synthesis of SHBG, TBG and CBG, (3) displace T_4 from TTR, (4) impair thyroid hormones metabolism in *the periphery*, (5) disrupt the *action of thyroid hormones* by sharing structural similarity with T_3, and (6) induce a 'net' TSH release from the pituitary. ER: estrogen receptor, TR: thyroid receptor, GR: glucocorticoid receptor, GnRH: gonadotrophin-releasing hormone, TRH: thyrotropin-releasing hormone, CRH: corticotropin-releasing hormone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, TSH: thyroid-stimulating hormone, FT_3, FT_4: free thyroid hormones, SHBG: sex hormone-binding globulin, TBG: thyroid-binding globulin, CBG: cortisol-binding globulin, TTR: transthyretin

receptors mimicking the TH activity so that the HPT axis does not perceive any reductions of TH levels and, therefore, no increase in TSH levels ensues.

An alternate or additional mechanism may involve TSH release from the pituitary [98]. Intracellular Ca^{2+} is known to regulate a large number of cellular functions including hormone secretion. PCB 95 has a direct and potent activity toward the ryanodine-sensitive Ca^{2+} release channels (RyRs) in the skeletal and smooth muscles [132] by targeting one of the RyR accessory protein complexes, FKBP12/RyR [133]. Influx of Ca^{2+} ions is necessary for the contraction of microfilaments and discharge of TSH-containing granules from the anterior pituitary gland [130]. In support to this, in PCB 95-treated rats, thyrotropin releasing hormone (TRH)-stimulated serum TSH levels increased only at 40% the control response to TRH suggesting that these congeners affect TSH secretion after TRH stimulation [134]. Interestingly, when adult male rats were administered a potent coplanar (non-ortho) PCB, 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) Fisher et al. reported a modest decline in weight gain, an induction of hepatic EROD and UDPGT activities, an increase of serum TSH levels, and a decrease of serum T_4 and free T_4 (FT_4) levels [135]. There is no doubt that HPT is altered by PCBs administration in more than one ways. Thus, secretion of TSH is not affected exclusively by the inhibition of TH binding to TTR (Fig. 3(1)).

Furthermore, in an experimental in vivo study, serum FT_4 levels in rats given the pesticide *N′N*-methylene-bis for 3 days significantly decreased, while TSH levels significantly increased. Histopathologic examination showed thyroid gland proliferation of follicular epithelium. It seems that the combined use of serologic test and histopathologic examination for screening TH disruptors may be an effective method [136]. In addition, lactational but not in utero exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of Holtzman rats resulted to a significant decrease in serum total T_4 and FT_4 levels in both sexes and a significant increase in serum TSH levels. UDP glycosyltransferase 1 family, polypeptide A6 (UGT1A6) and UGT1A7 mRNAs were induced in their livers [137].

Thyroid hormone transport proteins buffer against any sudden change in the availability of "free" plasma T_3. It is hypothesized that changes in the plasma levels of these transport proteins or competition of the binding sites by endocrine disruptors can alter the dynamic equilibrium between the "bound" and "free" plasma TH. The suggested mechanism by several, mostly animal, studies indicates that binding of thyroid disruptors to transport proteins might at first displace TH increasing, thus, their "free" fractions. This transitional state is rapidly followed by increased clearance of these hormones due to active glucuronidation of the increased FT_4 fraction [135], subsequently compensated by enhancement of TH production via HPT axis activation. The clinical result could be relatively increased TSH levels and/or thyroid gland enlargement (goitrogenesis) [130].

Interestingly, although in most animal studies involving endocrine disruptors, TH were found lowered, there is no clear evidence of TSH increase. Thus, the effect of endocrine disruptors in in vivo models is evident but not that important to involve a measurable HPT activation. Insight to this mechanism can be gained through the study of TTR-knockout animal models. These animals are euthyroid in the absence of this major plasma T_4 carrier [138]. The low T_4 levels found in the TTR-null mouse cannot be attributed to increased glucuronidation because liver UDP-glucuronosyltransferase activity is not affected [138]. Nevertheless, data derived from the study of knockout mice should be considered with caution because sometimes other homeostatic mechanisms compensate for the specific gene loss. Of note, TTR-depleted serum of wild-type animal shows increased T_4 binding to TBG.

Furthermore, there is evidence for TTR playing an important role in enabling T_4 transfer across the blood–brain barrier into the cerebrospinal fluid (CSF) of some mammals [139]. Since TTR represents up to 25% of the CSF proteins, it provides an abundant supply of T_4 to brain. Consequently, any condition or toxicant that inhibits T_4 binding by TTR in the CSF might restrict the availability of this hormone to the brain. TTR is described as only one factor of many determining TH distribution into the brain [140]. Thyroid hormones receptors expressed in brain regions mediate the TH effects [141]. By employing a TTR-null mouse, it was shown that TTR regulates "free" TH levels in the choroid plexus, but not in the brain parenchyma [142]. More precisely, it was found that (a) no other T_4-transport protein replaces TTR in the CSF of the TTR-null mice; (b) D2 activity is normal in the cortex, cerebellum, and hippocampus, and total brain RC3-neurogranin mRNA levels are not altered, and (c) T_4 levels measured in the cortex, cerebellum, and hippocampus are normal. In rats, the activity of brain D2 is markedly increased in thyroid hormone deficiency states maintaining, thus, adequate levels of in situ produced T_3 [143, 144]. Interestingly, experimental work has shown a markedly enhanced brain D2 in fetal and neonatal rats born to females that had been treated with PCBs [145]. Therefore, it seems that in late gestational fetuses, the induction of this enzyme compensates for decreases in brain T_4 levels [131]. A decrease in the clearance rate of T_3 by reduction in D3 expression may be another compensatory response to the decreased delivery of the T_4 'prehormone' to the brain [144]. Of note, D3 is responsible for the increased clearance of T_3 in the brain [146].

T_3 receptor

Recently, Japanese researchers found that the organohalogen compounds 4,4′-diiodobiphenyl (DIB), nitrofen (NIP) and 1,2,5,6,9,10-hexabromocyclododecane (HBCD), widely used as brominated flame retardants and pesticides, act as endocrine disruptors, in vitro, by enhancing TR- and/or ER -mediated gene expression [147]. Iwasaki et al. have shown that very low doses of PCBs can potentially interfere with TR-mediated transactivation by suppressing TR/SRC-1 co-activator complex [148]. Since SRC-1 coactivator gene is abundantly expressed in rat cerebellum during the developmental period known to be TH sensitive for the neonatal rat, PCBs may act directly on the central nervous system to suppress TR/SRC-1 coactivator interaction regardless of their binding to TTR [139, 143–145, 147–150].

Furthermore, some PCB congeners are potential candidates for interaction with TR, producing TH-like effects without causing apparent changes in TSH concentration [98]. Likewise, the removal of one or two ethyl groups of amiodarone results in compounds structurally similar to T_3, e.g., desethylamiodarone, being twice as potent in inhibiting the binding of this hormone to its receptor in vivo [151]. Among environmental chemicals that bind to TR and alter thyroid hormone signaling, the estrogenic compound bisphenol-A was reported to act as a TH antagonist on the TRβ in rats, resulting in increased total T_4 in nursing rat pups. The resulting hormonal profile is reminiscent of TH resistance syndrome [152].

In mice, 2, 3, 7, and 8-tetrachloro dibenzo-p dioxin (TCDD)-mediated myelotoxicity was instantly ameliorated after T_4 administration suggesting TCDD competition for binding with TR [153]. DDT toxicity in spotted munia, with regard to growth development, blood hemoglobin content, and plasma protein and cholesterol concentrations, was regulated by treatment with T_4 as well [6, 154].

OH-polybrominated diphenyl ethers (PBDEs) show structural similarities to TH. They bind with TR and serum TH-transport proteins [155]. In addition, PBDEs act as estrogen disruptors; several pure PBDE congeners as well as OH-PBDE are agonistic of both alpha- and beta-ERs and stimulate ER-mediated luciferase induction in vitro. Thus, PBDEs may produce potent pseudo-estrogens in vivo that can compete with TH for binding to TH-transport proteins (i.e., TTR) [156] (Fig. 3). Other ER-mediated pathways affecting testis development, hepatic enzyme activity, and behavior may also be affected [157, 158].

Endocrine disruptors and thyroid hormones metabolism

Experimental studies suggest that some types of TH deiodinases are strongly inhibited in the presence of environmental substances. Raasmaja et al. reported that TCDD administration to Long-Evans rats decreased D1 activity in the liver and increased D2 activity in the brown adipose tissue. TCDD also reduced serum total T_4 without affecting T_3. These effects of TCDD on 5′-deiodinases and TH are compatible with partial hypothyroidism. It is not clear whether TCDD affects the TH status and 5′-deiodination directly or through feedback mechanism. It seems that TCDD affects thyroid status in a tissue-specific manner while its effects on TH metabolism are more complex than just its influence on deiodinases [159]. Recently, the same authors reported that the changes of deiodinases activities are secondary to the decrease of T_4 levels [160]. Similarly, treatment of Sprague-Dawley male rats with the estrogen-like insecticide methoxychlor decreased hepatic D1 activity [161]. At the same time, Chaurasia et al. showed that lead inhibited hepatic D1 activity and thyroid function in cockerels, either by replacing selenium in the enzyme or by binding with the free-SH (sulfhydryl) groups, which are essential for the enzyme function [162]. Earlier reports on cadmium and mercury-induced-D1 inhibition in rats also supported these findings [162, 163]. Yoshida et al. (1987) showed that cadmium, either added to the incubation medium of the in vitro enzyme assays, or administered to rats prior to the experiments, reduced D1 activity resulting to reduction of hepatic T_3 production from T_4 [163]. Deiodinase enzymes require a thiol source as an electron donor, which in the case of the in vitro assays is dithiothreitol. Yoshida maintained that the effects of cadmium were exerted via interaction with dithiothreitol, possibly inhibiting this electron transfer metabolic pathway [163]. Apparently, more studies are needed to clarify whether 5′ deiodination is affected by disruptors. It is possible that changes in deiodinase activity might influence peripheral homeostasis of TH eventually participating in the development of a ‘euthyroid-sick’-like syndrome. The study of deiodinases knockout mouse models might help in the understanding of the extent of their action in TH homeostasis. Four mouse models have been described with varying deficiencies in the activating deiodinases: (a) D1KO mice; (b) D2KO mice; (c) C3H mice, and (d) C3H/D2KO mice [164–168]. Remarkably, each of these animals keeps normal serum T_3 concentrations and increased serum T_4 concentrations indicating that potent compensatory mechanisms exist to assure normal serum T_3 levels. One possibility is that high serum T_4 concentrations provide sufficient substrate to compensate for the loss of deiodinases activities. This T_4 levels increase may result from increased thyroid secretion and/or decreased clearance eventually as a result of the adaptation of the HPT axis to the loss of deiodinase activities [169]. More studies taking into account the dose-effect behavior of endocrine disruptors are needed to clarify the consequences of impairment of these enzymes activities on the HPT homeostasis.

Agents widely used in clinical practice, such as glucocorticoids, amiodarone, and propranolol, can alter TH metabolism. These compounds decrease the serum T_3 concentration, usually with a concomitant increase in the rT_3 level [97]. Drugs acting *via* acceleration of deiodination are diphenylhydantoin and phenobarbital, while cholestipol, ferrous sulfate, aluminum hydroxide, and sucralfate act *via* T_4 catabolism (glucuronidation) [97]. In the absence of inherent abnormalities of TH secretion or their regulation, the HPT axis will achieve a new equilibrium and TSH levels should return to normalcy [97].

Endocrine disruptors and thyroid tumorigenesis

Many goitrogenic substances exert a direct and/or indirect effect on the thyroid gland by disrupting certain steps in the biosynthesis, secretion, and peripheral metabolism of TH. Such substances are thiocyanate, perchlorate, sulfonamides, thiourea, methimazole, lithium or excess iodide. Amiodarone and iopanoic acid decrease peripheral T_3 production by inhibiting the deiodination process, which results in a compensatory increase of TSH secretion, follicular cell hypertrophy, and hyperplasia, and an increased incidence of follicular tumors in 2-year or lifetime studies in rats [170]. Epigenetic mechanisms interfering with intra- and extra- thyroidal sites of action were reported for numerous pesticides, as is the case with lofentezine, fenbuconazole, fipronil, mancozeb, acetoclor, thiazopyr, and toxaphene [171].

The crucial steps in thyroid follicular cell carcinogenesis in rodents include mutagenicity or perturbations of thyroid and pituitary hormone status with increased stimulation of thyroid cell growth by TSH, or both [172]. It is not clearly established whether chemicals that affect thyroid cell growth lead to human thyroid cancer. A useful line of investigation would be to compare susceptibility to disruptors-associated thyroid cancer between rodents and humans [173]. In experimental studies, thyroid is a frequent target for carcinogenic pesticides. Risk assessment requires evaluation of mode of action, mutagenicity, follicular cell growth and thyroid gland weight, thyroid-pituitary hormones levels, the site of action, correlation between doses producing thyroid effects and cancer, and reversibility of effects when the aggressor ceases to act. Ten percent of pesticides screened for carcinogenicity by the Environmental Protection Agency [174, 175] produced thyroid follicular tumors in rodents. However, mutagenicity seems to be attributable only to Acetoclor [171]. Regarding pesticide responses, amitrole, ethylene thiourea, and mancozeb induce a high incidence of adenomas, carcinomas, and combined adenomas/carcinomas in male rats. Conversely, bromacil, ethiozin, ethofenprox, fipronil, thiazopyr, pronamide, prodiamine, and pyrethrins, when administered at higher doses, induce lower thyroid tumor incidence in rats [173].

Endocrine disruptors and feto-maternal homeostasis of thyroid hormones

Thyroid hormones physiology during pregnancy

Maternal thyroid function is altered during gestation, with the rise of TBG being the most significant change. As a consequence, a new hormonal equilibrium is established via an increase of total T_3 and T_4 resulting in stable levels of "free" TH [176, 177]. There is usually no change in TTR and little change in albumin serum levels. Iodide clearance is increased. Thus, in areas of low iodide uptake thyroid pathology might ensue. Ionized iodide of maternal origin is transferred across the placenta in order to meet fetal needs. If the transported iodide quantities are increased, they may inhibit fetal thyroid function.

Thyroid hormones biosynthesis in the embryo starts between the 10th and 12th week of gestation [178, 179] (Fig. 2). During this period, fetal development depends upon maternal TH [177]. In 1989 Vulsma et al. proved, by observing embryos with a complete inability to iodinate thyroid proteins, that maternal circulation provided the embryo with 20–44% the normal levels of T_4 [180]. Nevertheless, the transplacental transport of maternal TH in the case of agenesia does not compensate for the absence of fetal TH and thus neurological abnormalities are not avoided. Maturation of the HPT axis occurs at the 16th and 20th weeks of gestation [181] (Fig. 2). Thyroid hormone receptors appear in the fetal brain between the 8th and 10th week of gestation [182]. Maternal hypothyroidism has been associated in some studies with mild cognitive impairment of the offspring [183–185]. In studies conducted in sheep, T_3 and T_4 metabolic compounds of fetal origin [e.g., 3,3′-diiodothyronine sulfate (T_2S)] are transferred oppositely via the placenta to maternal circulation. Since T_2S appears to be quantitatively derived from circulating T_3 in the fetus, a significant increase or decrease in T_2S in the maternal circulation would suggest hyper- or hypo- thyroidism in the fetus, respectively [186].

On the embryo TH contribute mainly to the development of the nervous system, the musculoskeletal growth, the maturation of the lungs, the genesis of the retina, and the development of the auditory pathway. Disturbance of the TH homeostasis causes neurological disorders of variable severity, which depend on the timing of the exposure at which they were induced (critical window) (Fig. 2), with the most important being endemic cretinism and congenital hypothyroidism. Other milder forms of disorders include

learning or memory disabilities, as well as attention deficit hyperreactivity syndrome in children [183, 187].

Thyroid hormone-disruptors and developmental disorders in the embryo

The theory postulating that the placenta represents a barrier to the toxic substances in maternal circulation has been set aside scientifically. Several exogenous substances, environmental or even pharmaceutical, have been shown to traverse the placenta, leading to severe medical problems to both the mother and the fetus [188]. During fetal and early neonatal periods, TH disorders may lead to the development of neuromotor and cognitive disorders. Animal models provide useful tools for the study of the effects of environmental endocrine disruptors on TH physiology during these periods of life. The congenitally hypothyroid, hyt/hyt mouse exhibits abnormalities in both the cognitive and motor systems [189]. The hyt/hyt mouse has a mutation on the TSH receptor gene resulting to a reduction of the TSH signal transmission into the thyrocyte [190]. Some behavioral and possibly some biochemical abnormalities in mice exposed to PCBs are similar to those seen in the hyt/hyt mouse, i.e., highest dose of PCB 126 led to increased serum TSH and decreased serum T_4 and FT_4 levels in adult male Sprague–Dawley rats [135]. Furthermore, interaction between endocrine disruptors and TR directly and/or indirectly involved in gene transcription could result to nervous system disorders such as impaired spatial learning and memory function. In analogy, pathologic phenomena such as severe anxiety and morphological changes in the hippocampus are also seen in TRα1-knockout mice [191].

The main categories of thyroid hormone-disruptors studied for their in utero effect are listed below.

Polychlorinated biphenyls

This is a family of substances mainly represented by PCB77 and PCB Aroclor1254. In 1998 Brouwer et al. found that a hydroxylated metabolite of PCBs, 4-OH-pentaCB, resulting from hepatic metabolism, shows increased affinity for TTR. This metabolite antagonizes FT_4 transport through the placenta to the developing brain of the embryo *via* binding to TTR. As a result, T_4 and FT_4 levels in fetal plasma and brain are reduced [148]. Taking into account that a substantial part of T_4 is of maternal origin [192], and decreased or low-normal maternal FT_4 levels during the first trimester seem to be associated with impaired psychomotor development of the child [193], maternal TH status and the amount of T_4 transferred across the placenta during perinatal exposure to PCBs should be further studied [194]. Interestingly, no significant TH abnormality was found in 160 children born between 1978 and 1982 in North Carolina and in utero exposed to PCBs [195]. In another major study, in 1996 Jacobson et al. reported neuromuscular and neurological developmental delay in the first 24 h of postnatal life in neonates whose mothers consumed for six or more years 2–3 meals of fish per month from the Great Lakes area (Michigan) contaminated with quantities of PCBs slightly higher than those in the general population. Also, a lower intelligence quota was reported in children of 4 to 11 years of age, prenatally exposed to PCBs [196]. In 1996 Koopman-Esseboom et al. diagnosed in infants up to 3 years of age neuromuscular disorders associated with in utero and lactational exposure to PCBs [197]. Furthermore, in a prospective study of 171 mother-infant pairs with prenatal, perinatal, lactational and postnatal (up to 42 months of age) evaluation of PCBs, a negative association between milk PCB and mental/motor development at all studied ages was found, becoming significant from 30 months onwards [198].

Thyroid hormone replacement therapy postnatally in rats, exposed in utero to these substances, attenuated hearing loss and motor deficits, suggesting that these developmental defects are mediated by PCB-induced T_4 decrease [199–202]. An additional action of T_4 (but not T_3) replacement in young rats perinatally exposed to Aroclor 1254 is the return of the PCB-depressed activity of choline acetyltransferase (ChAT) in hippocampus and basal forebrain toward normal [203]. Moreover, intrauterine exposure of monkeys to PCBs induced disorders of attention and combinative thinking [204], whereas mice exposed during intrauterine life to PCBs demonstrated a hyperreactivity syndrome [205].

Polybrominated diphenyl ethers

These chemical substances are used in industry for manufacture of plastic products. The exposure of mice to low doses during the vital phases of brain growth (intrauterine and neonatal period) induces irreversible changes in the mental functions of the adult mice [206]. Since TH regulate the development of cholinergic and dopaminergic systems serving the cerebral cortex and hippocampus, exposure to PBDEs during the developmental period might influence these systems by interfering with TH physiology [202], (i.e., decrease of hippocampal cholinergic receptors, as is the case in PBDE 153-exposed adult mice) [207, 208]. Recent evidence suggests that intrauterine exposure of Long Evans rats to PBDEs resulted in a decrease of serum T_4 and in the induction of phase-1 and phase-2 hepatic enzyme systems ethoxy- and pentoxy-resorufin-*O*-deethylase (EROD, PROD) and UDPGT. These enzymes are

implicated in TH catabolism in both rats and offspring that have been exposed in utero to PBDEs [209]. PBDE congeners, like their structurally similar PCBs, are known to displace T_4 from TTR [209].

Phenols: Bisphenol-A (BPA), pentachlorophenol (PCP)

Feeding pregnant rats with BPA results in significant increase of T_4 at postnatal day 15 in rats while serum TSH did not differ from controls [152]. Human literature on these compounds is sparse. In human newborns, PCP in cord plasma was negatively correlated to T_3, FT_4 and TBG [210]. These results suggest that PCP may alter TH levels in newborns and consequently may lead to adverse neurodevelopmental defects [211]. Pentachlorophenol, which demonstrates twice the affinity of T_4 to TTR, penetrates into fetal circulation and disrupts local TH supply in the developing human fetus [212].

Phthalates and plasticisers

There is not enough evidence or studies for the role of in utero exposure to these chemicals. Recently, it has been suggested that they may indirectly lead to thyroid function dysregulation in humans [212]. The authors demonstrated stimulation of Na^+/I^- symporter gene transcription by di-isodecyl phthalate (DIDP), benzyl butyl phthalate (BBP) and di-octyl phthalate (DOP). As a result, even permanent effects might ensue on the development of the central nervous system if changes occur in a critical developmental phase.

Pesticides (Nitrofen, DDT metabolites)

The pesticide Nitrofen induces lung hypoplasia in rat fetuses when administered to the mother during gestation. This effect is achieved by a non-competitive inhibition of the binding of T_3 to TRα1 and TRβ1 receptors [213]. In addition, Nitrofen downregulates thyroid transcription factor-1 (TTF-1) both in vivo and in vitro [214]. Thyroid transcription factor-1 is a homeodomain transcriptional factor expressed in thyroid, lung, and parts of the brain. In vitro, TTF-1 can activate the promoter of thyroid- and lung- specific genes [215]. Early in fetal development, TTF-1 regulates branching morphogenesis and proximal-distal patterning in the lung. Alterations in TTF-1 during gestation affect lung maturation, both architecturally and biochemically as it is the case in the rodent model of Nitrofen-induced congenital diaphragmatic hernia [216].

In another study, plasma levels of three pesticides (*p,p′*-DDE, *cis*-nanochlor and hexachlorobenzene) were found to have a significant negative correlation with maternal T_3 levels without any other changes in the thyroid status [217]. A more recent study demonstrated that exposure to DDE and its metabolites during fetal development is negatively associated with cord serum T_4 levels in infants, emphasizing the need to further investigate the adverse effects on thyroid hormones, growth, and neural development in children earlier exposed to high doses of DDT [218].

Dioxins [polychlorinated dibenzo-p-*dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs)]*

Infants exposed prenatally to higher toxic equivalence levels of dioxins and dibenzofurans had elevated levels of TSH both two weeks and three months after birth, and lower levels of FT_4 and T_4 two weeks after birth [219]. The TH alterations observed in these infants are, however, within the normal range of clinical values. In contrast to previous studies no effects on the cognitive development of these infants at three or seven months were reported. Whether this discrepancy should be attributed to differences in levels of exposure it remains to be determined. A major case of prenatal poisoning with PCDFs occurred in 1968 in Yusho, Japan, due to the consumption of contaminated rice oil. Dramatic adverse effects were observed in babies born to exposed women, including low-birth weight, skin discoloration, bronchitis, and developmental retardation [220]. Behavioral effects in the Yusho infants included hypoactivity and hypotony [221, 222]. The intrauterine exposure to dioxins causes a significant degree of disorder in thyroid function and the development of the newborns [223]. Recently, it has been reported that maternal exposure to increased concentrations of dioxin-like compounds was associated with incidence of congenital hypothyroidism [224].

Another dioxin, TCDD, the most toxic among PCDD/F congeners, when given to pregnant rats was transferred to their offspring *via* transplacental and lactation routes [225]. Administration of TCDD to rats during gestation was correlated with T_4 decrease and a twofold increase of TSH of the male offspring as well as hyperplasia of the thyroid gland [226].

Conclusions

The delivery of the adequate TH quantity to target tissues, under euthyroid conditions, is the result of synthesis, transport, metabolism, and excretion of these hormones. Thyroid hormones homeostasis depends on the

maintenance of the circulating 'free' TH reserves. Disturbance of this hormone system, which is in constant interaction with other hormone systems, leads to an adaptational counter-response targeting reestablishment of a new homeostatic equilibrium. Endocrine disruptors are chemical substances forming components of 'natural' contaminating agents found in most ecosystems. Most of the data investigating the effects of endocrine disruptors on thyroid physiology resulted from experimental studies performed in rodents and some in humans. Endocrine disruptors target the HPT axis at several levels including Na^+/I^- symporter, deiodinases, transport proteins, and TR. Thyroid hormone receptor or deiodinase knockout transgenic mouse models might help in the understanding of the endocrine disruptors influence on the HPT axis. Till now, a substantial amount of information, although not conclusive, has been gathered regarding the way HPT axis is affected by the endocrine disruptors action. Furthermore, it is evident that more than one endocrine axis are affected depending that much on the structure of each endocrine disruptor (qualitative property) as much on its dose effecting on an intact endocrine system (quantitative property). Estrogonoids, such as DDE, DDT, Bisphenol A besides affecting genital tract development, alter "free" TH levels by increasing T_4-binding to TBG.

During fetal life, TH contribute to the process of differentiation of tissues. Decreased availability of these hormones in utero results in irreversible developmental disorders of the fetus. The exposure of thyroid physiology to endocrine disruptors at critical periods of fetal development may cause permanent structural and functional brain abnormalities with postnatal behavioral changes. Concerning the prenatal and perinatal (mostly lactational) exposure to disruptors, it is quite obvious that single-time measurements approach during pre- or post-natal period, cannot test the cumulative or synergistic property of these substances. However, substantial part of the T_4 circulating in the fetus is of maternal origin and action of chemicals on maternal TH status seems to be associated with impaired development of the fetus. Moreover, a thorough investigation should combine data with the maternal physiological changes that take place during pregnancy aiming to meet the needs of the conceptus in terms of energy supply and waste elimination. With regard to TH physiology, the three compartments met during pregnancy (maternal, placental, and fetal) interact closely and they should be approached as an entity.

Given the scarcity of human data and the insufficient iodine-supply to almost one third of the world population, epidemiological studies should be supported by efficient in vitro assays. The latter have been already proved useful in assessing the adverse effects of thyroid disruptors and could help the accomplishment of a toxicity-based classification. At present, the action of the scientific community must be extended to two levels: (a) the limitation of exposure to endocrine disruptors, and (b) the detailed study and evaluation of the dose–response 'behavior' of the impairment of thyroid gland function as well as the correlation of the toxicity of endocrine disruptors amongst species through interdisciplinary studies. Nevertheless, the human being must ultimately choose between the short-term benefits that technological advances offer and the global quality of life based on respect for the environment, wildlife, and humanity. Further studies should focus on specifying the HPT axis adaptational changes when thyroid-disrupting chemicals challenge the organism.

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