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Endocrine disruptors and thyroid hormone physiology

Mary-Line Jugan[a]*, Yves Levi[a], Jean-Paul Blondeau[b]

[a]Univ Paris-Sud 11, Faculté de Pharmacie, Laboratoire Santé Publique-Environnement, 92290 Châtenay-Malabry, France

[b]Univ Paris-Sud 11, Faculté de Pharmacie, CIBLOT platform, 92290 Châtenay-Malabry, France

* Corresponding author at: Laboratoire Santé Publique-Environnement, Faculté de Pharmacie, rue J.B. Clément, 92290 Châtenay-Malabry, France.

Tel.: +33 146 835 857;

E-mail address: maryjugan@yahoo.fr (M.L. Jugan).

Abbreviations: T3, triiodothyronine; T4, thyroxine; TH, thyroid hormone; TSH, thyrotropin; CAR, constitutive androstane receptor; PPAR, peroxisome proliferator-activated receptors; PXR, pregnane X receptor; RXR, retinoid X receptor; TR, thyroid hormone receptor; N-CoR, nuclear corepressor; SRC, steroid receptor coactivator; SULT, sulfotransferase; UGT, glucuronosyltransferase; BBP, butylbenzyl phthalate; BPA, bisphenol-A; DBP, dibutylphthalate; DCHP, dicyclohexylphthalate; DEHP, di(2-ethylhexyl) phthalate; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; TBBPA, 3,3',5,5'-tetrabromobisphenol-A; TCBPA, 3,3',5,5'-tetrachlorobisphenol-A; TDC, thyroid disrupting chemical.

ABSTRACT

Endocrine disruptors are man-made chemicals that can disrupt the synthesis, circulating levels, and peripheral action of hormones. The disruption of sex hormones was subject of intensive research, but thyroid hormone synthesis and signaling are now also recognized as important targets of endocrine disruptors. The neurological development of mammals is largely dependent on normal thyroid hormone homeostasis, and it is likely to be particularly sensitive to disruption of the thyroid axis. Here, we survey the main thyroid-disrupting chemicals, such as polychlorinated biphenyls, perchlorates, and brominated flame retardants, that are characteristic disruptors of thyroid hormone homeostasis, and look at their suspected relationships to impaired development of the human central nervous system. The review then focuses on disrupting mechanisms known to be directly or indirectly related to the transcriptional activity of the thyroid hormone receptors.

Keywords: thyroid disrupting-compounds, thyroid hormone receptors, polychlorinated biphenyls, flame-retardants, neurodevelopment, endocrine disruptors

1. Introduction

The term endocrine disruptor is used to describe substances that can potentially interfere with the endocrine system. In vivo and in vitro studies initially focused on their estrogenic, anti-estrogenic or anti-androgenic effects on wildlife, experimental animals, and humans, but more recently, their effects on thyroid signaling have also received attention. The thyroid hormones (THs), thyroxine (T4) and triiodothyronine (T3), are critical in regulating the growth and differentiation of many tissues and organs, as well as energy homeostasis and numerous key metabolic pathways. THs are known to play an important role in the perinatal development of the central nervous system [1]. This means that disruption of the TH axis can cause severe impairment, particularly of mammalian brain maturation, resulting in mental retardation and neurological defects. There is currently considerable concern about a potential relationship between the increasing prevalence of neurodevelopmental disorders, and the almost exponential increase in exposure to pollutants over the past fifty years. This relationship was discussed for the first time in 2004 by Colborn [2], who highlighted the difficulty of establishing this causal link, and the need to develop improved tools to detect and assess thyroid-disrupting chemicals (TDCs). Since then, epidemiological data have accumulated, and many tools have been developed to detect and elucidate the mechanism of action of TDCs. In this review, we focus first on the main environmental chemicals known to disrupt TH physiology in humans, and to a lesser extent in other species. We then concentrate on the molecular mechanisms responsible for thyroid disruption, particularly those implicating the thyroid hormone receptor related pathway.

2. Thyroid-disrupting chemicals

It is difficult to demonstrate any causal link between pre- or post-natal pollutant exposure and developmental adverse effects in humans. Nevertheless, recent well-designed cohort studies supported by laboratory animals and in-vitro research have highlighted several classes of chemicals that are particularly suspected of exerting adverse effects on the human thyroid axis. The four compounds selected in this review had been suggested in recent publications as being suspected endocrine disruptors. They also have been included in the priority list of chemicals established by the European Union and US-EPA, within their strategies for endocrine disruptors assessment [3-4].

2.1. Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are an important group of persistent man-made contaminants. Use of these compounds has been proscribed since the 1980s, but PCBs are still frequently detected in human tissues, including breast milk, the umbilical cord, and blood [5]. In terms of human health, pre- and post-natal exposures to PCBs are often associated with mental retardation and neurological defects in exposed children [6]. Several epidemiological studies have demonstrated the existence of links between PCB exposure and neurodevelopment impairment (Table I: 7-10;13). However, the statement concerning adverse effects of PCBs is discordant (11-12).

Most of studies reported significant effects on children after a prenatal exposure (Table I). Boucher et al. [14] recently published a meta-analysis, in which they

attempted to identify the cognitive functions that are particularly susceptible to prenatal exposure to PCBs. They reported that the most consistent effects observed in these studies are impaired executive functioning related to increased prenatal PCB exposure. The other main adverse effects affect processing speed, verbal abilities, and visual recognition memory, and are also often reported.

PCB levels detected in human blood nowadays are markedly lower than in the 1970s [15]. Interestingly, Wilhelm et al. [11] compared mental and motor development in newborns from two cohorts with different prenatal exposure patterns in the same geographic area. The Dusseldorf cohort study, carried out in the 1993-2000 period, revealed an adverse impact of prenatal PCB exposure on the neurodevelopment of children at the ages of 12 and 24 months. Another newborn cohort study was initiated in 2000 in the industrial city of Duisburg, located 30 km from Dusseldorf. This study demonstrated that prenatal exposure was about two- to threefold lower than in Dusseldorf, and that prenatal PCB exposure did not impair neurodevelopment in the children in the Duisburg cohort. The evidence suggests that, in Germany, exposure to PCBs at current exposure levels apparently no longer impairs neurodevelopment in children.

Reports of the adverse effects of PCBs in the context of postnatal exposure are more inconsistent. A few authors have observed adverse effects due to lactational exposure [7-8], whereas other studies have not confirmed this association ([9,11-12]).

Furthermore, the mechanisms implicated in the adverse effects of PCBs on neurodevelopment are not clear. Several authors have suspected that these effects might be mediated by disruption of TH homeostasis [16]. Table II summarizes various cohort studies in which PCB exposure was correlated to a disruption of

circulating levels of THs in humans. In several studies, environmental PCB levels were shown to be associated with reduced TH levels [17-18, 20].

There are fewer reports of a positive correlation between PCBs and TH levels [19, 21-22]. Dallaire et al. [21] have published an interesting study, in which PCB exposure was evaluated in different matrices including maternal plasma during pregnancy, the umbilical cord blood at birth, and the offspring's blood at 7 months. Hydroxylated PCB (OH-PCB) concentrations were positively associated with T3 concentrations in the plasma of pregnant women, whereas PCB-153 concentrations were inversely related to thyroxine-binding globulin levels in umbilical cord blood, whereas no correlation was observed in children's blood at 7 months of age. Potential thyroid toxicants did not have the same effects on TH homeostasis in mothers and their infants. This study highlights the importance of taking different exposure matrices into account, and of carrying out long-term follow-up determinations at different ages.

Most of the cohort studies mentioned above deals with the association between PCBs and motor/mental development, or between PCB and TH status. However, little data is available that reports the correlation between all three parameters (PCB, TH levels and neurodevelopment) in humans. As far as we are aware, only one study has dealt with this association. The Duisburg birth cohort study did not exhibit any correlation between these three parameters in human subjects [12]. As mentioned above, the human biomonitoring results of this study underlines that the PCB exposure has decreased compared to the exposure reported in the cohort studies of the last 10–15 years. Therefore, no associations between exposures, disruption of the levels of THs, and neurological and developmental measures were observed. There is a real need to develop this kind of approach in

areas where the levels of exposure are higher, in order to provide stronger evidence that thyroid dysfunction induced by PCBs can cause neurological abnormalities in human. Moreover, it appears to be important to take into account potentially critical toxicokinetic parameters, such as absorption, bioaccumulation, metabolism, and excretion, in order to provide a better understanding of the relationship between PCB exposure and adverse neurodevelopmental effects. It is also important to investigate on the structure-activity relationship of PCBs, to determine the potential hazard of existing and future synthetic chemicals for the developing brain. Differences in PCB groups or congeners may explain the discrepancies that have been observed between existing studies. Some clues have suggested that prenatal exposure to dioxin-like PCBs is a cause for greater concern than non-dioxin like PCBs [13], and that hydroxylated PCBs, which display a high degree of structural similarity to thyroxin (T4), could be even more worrying.

Finally, these studies suggest that even at low levels of exposure, PCBs can interfere with thyroid hormone levels, particularly tending to lower them. Most physiologic functions, such as behaviour and processes involved in foetal development, are supported by multiple endocrine axes that may communicate with each other. Hence, even when a chemical affects a physiologic endpoint, it remains difficult to prove that this effect results from disruption of a particular endocrine system. Although consistent hypothyroid effects have been found in association with PCB perinatal exposure, a causal link in neurodevelopmental dysfunction cannot be unequivocally deduced from these human data. The hypothesis that neurotoxic effect of PCBs on brain development is probably caused by hypothyroidism is predominant in the literature, a theory that is strengthened by several in vivo experiments. Interestingly, Goldey et al [23] showed that T4 replacement attenuated the

hypothyroxinemia, hearing loss and motor deficits due to developmental exposure of rats induced by a mixture of PCBs (Aroclor 1254). Indeed, several studies have established a causal relationship between PCB exposure and decreased T4 levels in rats [24] and monkeys [25].

2.2. Perchlorates

Perchlorates occur both naturally and through manufacturing, for use by the aerospace, weapons, and pharmaceutical industries. These compounds are extremely water-soluble, and have been detected in rain, snow, foodstuff, ground waters, and fertilizers. They have also been detected in U.S. drinking water supplies at levels ranging from 4 to 200 microg/liter [26]. Perchlorates are competitive inhibitors of iodide transport into thyroid follicular cells, and are established inhibitors of TH synthesis [27]**.** This property is the basis of its use in the diagnosis of TH synthesis dysfunction and the treatment of hyperthyroidism.

Whether circulating TH and TSH concentrations are influenced by perchlorate exposure is a matter of debate in the scientific literature. Blount et al. [28] established a relationship between the presence of perchlorates in blood and urine, and TSH (positive association) and total T4 (negative association) levels, particularly in women with a urinary iodide level < 100 μg/L. The authors pointed out that this group was more susceptible to the competitive inhibition of thyroid iodine uptake by perchlorates than women with urinary iodide > 100 μg/L, for whom no correlation was found between T4 level and perchlorate exposure. As the relationship between perchlorate exposure and T4 concentration can be modified by the individual iodide status, combined exposure to thiocyanates (in tobacco smoke) can also interfere with this

relationship [29].

In contrast, no such a correlation was established for men. This observation is in line with other cohort studies [30-31]. For example, Lawrence et al. [30] demonstrated the sensitivity of the thyroid iodide trap to perchlorate in nine healthy men who were daily administered a dose of 10 mg perchlorate daily for 2 weeks, but also found that circulating concentrations of TH and TSH were not affected. Braverman et al. [32] observed that a 6-month exposure to perchlorate at doses up to 3 mg/day had no effect on thyroid function in 13 healthy volunteers. The parameters studied were the inhibition of thyroid iodide uptake and the serum levels of thyroid hormones, TSH, and thyroglobulin. High doses and long-term exposures have also been investigated in the context of occupational exposure. Braverman et al. [33] demonstrated that as a result of high absorption while exposed during three night's work the iodide uptake was 38% lower than in unexposed workers. However, neither serum TSH and thyroglobulin concentrations nor thyroid volume were affected by perchlorates, suggesting that long-term exposure to high levels of perchlorates did not induce hypothyroidism or goiters in adults.

Taken as whole, human studies tend to suggest that perchlorates exposure carries little risk in healthy subjects. Women appear to be more sensitive than men, but the reason for this difference is not clear. However, perchlorates are of concern in some susceptible subjects, particularly people with iodide uptake abnormalities. Experimental studies in animals provide stronger evidence that perchlorates could interfere with thyroid homeostasis, and may have adverse effects on neurodevelopment. Gilbert and Sui [34] recently reported the results of exposing pregnant female rats to perchlorates in drinking water in order to evaluate neurologic development in rat pups after in utero exposure. They found that the highest

exposure to perchlorate during development caused a reduction in T4 levels in pups on post-natal day 21. For example, T4 was 16%, 28%, and 60% lower than control in the 30-, 300-, and 1,000-ppm dose groups, respectively, and this reduction was associated with an increase in TSH in the high-dose group. Whereas motor activity and spatial learning were not impaired, developmental exposure to perchlorate induced neurologic damage, as this compound did alter synaptic transmission in the hippocampus of the adult rats. Nevertheless, rats were shown to be more sensitive to perchlorates than any of the other tested species including humans, mice, and rabbits. Furthermore, McNabb et al. [35] reported disruption of TH homeostasis in birds exposed to perchlorates in drinking water. Birds exposed to low doses (5 mg/L for 8 weeks) exhibited a decrease in serum T4 during the two first weeks of exposure. Interestingly, T4 levels had recovered after an 8-week exposure. This initial decrease was associated with an increase in TSH levels, suggesting that compensatory mechanisms were at work. This adaptation is not observed in adult birds. This study illustrates the complexity of assessing thyroid-disrupting chemicals, as their effects depend on the duration and dose of the exposure, as well as on an exposure window.

2.3. Brominated flame retardants

Polybrominated diphenyl ethers (PBDEs) are used as flame-retardants in electronic equipment, textiles, and construction materials. Their structure, persistence, and bioaccumulative properties are similar to those of the PCBs. Over the last 20 years, human body burdens of PBDE have increased, whereas those of PCBs have declined [15]. These compounds have been detected in many environmental samples and in human blood [36].

The effects of PBDE on TH homeostasis have only been investigated in a few studies, as these compound are considered as emerging pollutants [37-38]. Two of these studies reported that PBDE exposure was associated with increased T4 levels [37, 38]. Julander et al. [37] reported a tendency for increased free plasma T4 in response to BDEs 28, 153, and 183 in electronic recycling employees. This association was also observed in men exposed to levels comparable to those confronting the general U.S. population, whose body burdens were consistently positively associated with their T4 level, including total T4, free T4, and urinary T4 [38]. This study, which is the only large study linking PBDE exposure to TH homeostasis, also demonstrated a negative association between PBDEs and T3 and TSH.

Although human health studies are very limited, PBDEs are of concern because in vivo studies demonstrate endocrine adverse effects. Biological adverse effects in animals seem to be similar to those of PCBs. Decreased T4 levels have been reported in birds, Xenopus laevis, fishes, and rodents [39-42]. Perinatal exposure of rodents to PBDEs has been shown to cause long-lasting changes in motor activity, usually described as hyperactivity, and to disrupt performance in learning and memory tests [43]. There is a lack of reliable human data that could be used to support these conjectures extrapolated from animal studies. As PBDEs are classified as emergent pollutants, it appears to be essential to deal with the possible effects of human PBDE exposure for future use.

2.4. Phthalates

Phthalates are man-made chemicals used to improve the flexibility of plastics. They have been used in various products, such as toys, medical tubing, plastic

bottles, packing cases, and cosmetics. European production of phthalates has recently been documented and is approx. one million tons per year [44]. Animal studies have shown that some phthalates, for example dibutylphthalates or di(2-ethylhexyl)phthalates, cause adverse male reproductive health outcomes [45].

Phthalates have no intrinsic anti-thyroid activity in humans, but a by-product generated by gram-negative bacteria is an inhibitor of thyroperoxidase, an enzyme strongly involved in TH synthesis. This mechanism probably explains the relationship between phthalate exposure via drinking water and the prevalence of goiters reported by Gaitan et al. [46]. A limited number of studies suggests that exposure to some phthalates may be associated with altered thyroid function, but human data remains insufficient. To the best of our knowledge, only two studies have investigated the association between environmental exposures to phthalates and serum thyroid hormone and TSH levels in humans. These studies both show that urinary levels of phthalates may be associated with altered free T4 and/or total T3 levels in adult men and pregnant women [47-48]. These studies highlight the need for additional research to confirm these findings. To our knowledge, no data on the consequences of animal exposure on thyroid functions and neurodevelopment is available.

To conclude, it is suspected that several groups of pollutants may disturb TH homeostasis. PCBs, perchlorates, and PBDEs are of particular concern, and illustrate the concerns related to establishing cause and effect relationships between environmental chemical exposure and biological hazards at the population scale. The disruption of TH homeostasis by xenobiotics often results in equivocal findings. Numerous hypotheses could account for these discrepancies, including the biological matrices used to characterize the exposure (blood, plasma, breast milk, placenta…),

and the contaminants that were measured (chemicals or metabolites). Concomitant exposure to other pollutants, crosstalk with other endocrine systems, and urinary iodine concentrations influences are not often taken into account, whereas these three parameters are of considerable importance. Considering the numerous evidence of TH disruption by pollutants in humans, and of adverse effects in animals, it is clearly important to carry out more well-designed human studies to confirm this cause-effect relationship, and to determine the risk of neurodevelopmental effects at current levels of exposure. There is an abundant literature on the mechanisms of action of TDCs. This subject is developed in the following section.

3. Mechanisms of action of TDCs

A major difficulty in studying thyroid disruption is the complexity of the mechanisms of action involved. The TH axis is rather complex, and thyroid-disrupting chemicals can target the thyroid endocrine cascade at various levels, including that of the synthesis of THs by the thyroid gland and its regulation by hypothalamic-pituitary hormones, the catabolism and clearance of circulating THs by the liver and kidneys, the binding to transport proteins in the circulation, the cellular uptake of THs, the peripheral activating/inactivating metabolism of THs by iodothyronine deiodinases, the transcriptional activity of TH receptors (TRs) and the expression of TH-regulated genes (fig. 1).

We reported in the previous section that TDCs are usually identified by their potential to affect circulating TH levels. Subsequently, attention has focused mainly on the aspects directly related to the effect of toxicants on hormone levels, e.g. thyroid histology, TH synthesis, transport in the blood, and catabolism. However, it is

now increasingly recognized that various chemicals can produce effects on TRs, and on their transcriptional activity [49]. Thus, it is conceivable that a TR-disrupting chemical exerts effects that mimic the action of THs or of TH insufficiency, in particular on brain development.

In fact, the THs influence a broad range of developmental processes during brain maturation, including the differentiation and migration of neural and glial cells, and myelinization. Several of the genes involved in these processes have been identified as being regulated by THs [50]. Gene regulation by THs in the brain displays strict temporal and regional specificity [1]. Disruption of transcriptional processes by exogenous chemicals can influence the sequence of TH-sensitive developmental events and lead to adverse effects. Investigations of TDC mechanisms that could involve non TR-related mechanisms are abundantly documented [51], and so we will now focus on TR-related mechanisms in the actions of pollutants.

3.1. Mechanisms of action of THs and potential transcriptional targets of TDCs.

T4 is synthesized in the thyroid gland and is less metabolically active than T3, which is formed in the thyroid gland and in peripheral tissues by specific enzymatic deiodination of T4. The biological actions of THs are driven by the binding of T3 to nuclear thyroid hormone receptors (TRs). These TRs are products of two genes that encode three major functional TR isoforms in humans: TRα1, TRβ1, TRβ2. TRα1 and TRβ1 are expressed in almost all tissues [52]. However, these two main isoforms have differing spatial and temporal expressions and are also differentially expressed during development. TRα is particularly abundant in the brain, heart, and immune

system, whereas TRβ1 is particularly expressed in the brain, liver, and kidney. The TRs belong to the nuclear receptor superfamily of ligand-inducible transcription factors. TRs form homodimers and also heterodimers with other nuclear receptors, in particular with the retinoid X receptor (RXR) [53]. These active forms bind to thyroid hormone response elements (TRE), which are usually located in the regulatory regions of target genes on nuclear DNA, and control transcription by interacting with coactivators and corepressors. TRs can be either T3-dependent or T3-independent transcription factors, depending on their interaction with transcriptional cofactors [54]. When unoccupied by T3, the TRs recruit basal repressors, such as the silencing mediator of the retinoid and thyroid hormone receptor (SMRT) and the nuclear receptor corepressor (NCoR). These cofactors allow the additional recruitment of histone deacetylases, which remodel the structure of chromatin, and induce the repression of target genes at lower levels than in the absence of the receptor, a status known as "basal repression" or "silencing". Conversely, T3 binding to the TRs induces conformational changes that result in corepressor release, and subsequent coactivator recruitment. These coactivators, such as those of the p160/SRC (steroid receptor coactivator) family, have inherent histone acetylase activity and initiate a molecular cascade that culminates in the activation of target gene transcription.

Environmental chemicals may target this TH-related transcriptional process at various levels (fig.2). In the last years, the number of published cellular assays dedicated to the detection of TDCs has increased considerably. Indeed, transactivation and proliferation assays are viewed as useful tools that can be used as a quick way to detect disrupting chemicals with thyromimetic or TH-antagonistic activities in environmental samples. Among the few standardized and validated tools that have been developed, the “T-screen” has been widely used [55]**.** This assay is

based on the TH-dependent proliferation of rat pituitary cells GH3. Transactivation tests have also been used to study the TR-mediated effects of TDCs. These tests are based on a luciferase reporter gene controlled by T3-responsive elements. To be a useful reporter gene for identifying and quantifying TDCs, a gene must satisfy several requirements, e.g. it must be stably transfected with the reporter system and display high sensitivity and low variability. Two stably-transfected luciferase reporter gene assays appear to be valuable tools with which to study chemical interference with the TRα1- and TRβ1-signaling pathways [56,57]. Although cellular tests do not provide any precise information about the specific molecular targets of pollutants in the TH-dependent transcriptional process, they do make it possible to discriminate between agonists, partial agonists and antagonists of the TR. Indeed, cellular tests can be performed both in the absence and presence of T3 in order to assess independently the agonistic and antagonistic effects of a chemical, respectively.

3.2. Are TDCs transcriptional effects mediated by the ligand-binding site of TRs?

A few environmental chemicals have been shown to bind to TRs, and to modulate its activity. Kojima et al. [58] compared the TH-agonist and -antagonist activities of the main PBDE congeners and their hydroxylated metabolites, using CHO cells transiently transfected with TRα1 or TRβ1 expression plasmids, and a luciferase gene controlled by a T3-responsive element. Among the 16 compounds tested, only 4-OH-BDE-90 displayed a weak effect in the cellular test. They found that a higher concentration (10^{-5} M) of 4-OH-BDE-90 significantly inhibited both the TRα1- and TRβ1-mediated transcriptional activity induced by T3, whereas its corresponding metabolites did not. Moreover, none of the PBDEs tested showed any agonistic

activity. Kitamura et al. [59] also demonstrated that four OH-PCB congeners (4-OH-2,2′,3,4′,5,5′-HxCB ; 4-OH-3,3′,4′,5-TCB ; 4,4′-diOH-3,3′,5,5′-TCB) activated T3-induced GH3-cell proliferation in the same range of concentrations. These studies suggested that the antagonist activity of OH-PBDE could be due to the 4-OH-PBDE binding to the rat TR. Indeed, Kitamura et al. [60] reported that OH-PBDE was bound to the rat TR. 4-OH-BDE-90 and 3-OH-BDE-47, like pentabromophenol, inhibited the binding of T3 in the concentration range of 10^{-6} M to 10^{-4} M. High binding affinity was observed, particularly for 3- and 4- hydroxylated PBDEs with bromine substitutions at both positions adjacent to the hydroxyl group. This structural factor seems to be a good predictor of a competitive inhibition mechanism, which could explain, at least partially, the antagonistic activity of OH-PBDEs at the TR. Finally, it has been reported that environmental chemicals can selectively discriminate between TRα and TRβ [61]**.**

In view of the structural resemblances between PCBs (and PBDEs) with thyroid hormones, the affinity of these chemicals for the TR has especially been investigated. Using a competitive binding assay, Kitamura et al. [59] reported that nine OH-PCBs, which have been detected as metabolites of PCBs in animals and humans, markedly inhibited the binding of T3 to the rat pituitary TR in the concentration range of 10^{-6}M to 10^{-4}M. In contrast, Cheek et al. [62] reported that human recombinant TRβ showed low affinity of for OH-PCB, which was 10,000-fold lower than its affinity for THs. Moreover, Fritsche et al. [63] used a model of normal primary human neural progenitor cells to find out whether PCBs interfere with TH-dependent neural differentiation. They identified one PCB (PCB-118), known to be a common contaminant of the human population, as being able to mimic the capacity of T3 to induce differentiation of neural progenitors cells. This effect was dose-

dependent between 0.01 and 0.1 microM, and was congener specific. Interestingly, PCB-118 activity was blocked by the TR antagonist NH-3, which indicated that this environmental compound mimicked the action of T3 by interacting with T3-binding site of the TR. The question of PCB structural factors that could preferentially have affinity for TR has also been studied by Arulmozhiraja et al. [64], who did not identify any significant correlation among the 91 PCBs tested. Gauger et al. [65] also showed that a large variety of PCBs, and their metabolites, did not competitively bind to TR.

To date few studies have investigated the effects of phthalates on the action of TH. The possible disruption of TH-related transcription by phthalates mediated by binding to TRs was mentioned by Ghisari et al. [66]. They used the T-screen to analyze a large range of widely-used plasticizers, including phthalates and phenols, and found that all the compounds tested stimulated GH3 cell proliferation. Moreover di(2-ethylhexyl) phthalate (DEHP), dibutylphthalate (DBP), dioctylphthalate and diisononyl phthalate all inhibited T3-induced cell growth. Unlike to other phthalates used in this study, DEHP was also shown to interfere with the binding of T3 to TR [67]. This compound could therefore compete with T3 binding to TR. Nevertheless, the antagonistic effects of other compounds remain unclear.

Environmental chemicals, particularly PBDEs, have been reported to bind directly to TRs [60]. As mentioned in the first section, most studies have investigated the effect of pollutants on TH circulating levels. Nevertheless, the impact of TDCs on ligand binding to the TR should not be underestimated, and is likely to produce adverse effects on the developing brain. However, their affinity is typically low, and alternative mechanisms of action may be involved in the disruption of TR activity.

3.3. Disruption at other levels of the transcriptional process

As described above, some thyroid-disrupting compounds mimic or antagonize the effect of T3 on the up-regulation of TH target genes. As only few compounds exhibit any affinity for TR, it is very likely that other mechanisms underlie the disruption of transcription. Several studies highlight the possibility that some TDCs may interfere with a process or several processes within peripheral target cells other than T3-binding to TR in the T3-signaling pathway. For example, using a stable reporter gene assay, Jugan et al. [57] reported that several halogenated phenolic compounds (3,3',5,5'-tetrabromobisphenol-A (TBBPA) and 3,3',5,5'-tetrachlorobisphenol A (TCBPA)) could mimic the ability of T3 to induce TRα1-dependent transcription, at micromolar concentrations. When added together with T3, TBBPA and TCBPA both behaved as inhibitors of T3-stimulated transcription. Interestingly, this inhibition was partially counteracted for TBBPA and TCBPA by an excess of T3, suggesting a partially competitive mechanism, which would be consistent with these compounds' having a weak affinity for TR [68]. However, non-competitive mechanisms are also implicated. The reporter gene assay was not able to specify the molecular mechanisms involved.

Furthermore, studies reported the potential of chemicals to mimic T3 activity on TR, without necessarily bind TR. TDCs could potentially exert their effects by promoting the recruitment of coactivators by TRs, or by inhibiting the process induced by T3. Furthermore, detailed kinetic studies, using the reporter gene technology, have suggested that some compounds, such as TBBPA, may induce corepressor release but not coactivator recruitment by TRα, resulting in the removal of reporter gene silencing (fig. 2). This pattern of cofactor disruption is reminiscent of

that of NH-3, a synthetic selective TRβ1 antagonist that inhibits TH action both in vivo and in vitro [69]. In fact, NH-3 inhibits both coactivator and corepressor binding, and appears to be an agonist in terms of corepressor release, and an antagonist in terms of coactivator binding. A few studies have reported that TDCs might disrupt the function of cofactors. In a transient reporter gene assay, Moriyama et al. [70] demonstrated that bisphenol-A (BPA), a chemical widely used in the production of plastics, suppressed TR-mediated transcription. They combined binding experiments and mammalian two-hybrid assays to demonstrate that BPA could impair TH action both by inhibiting T3 binding to TR and by recruiting the nuclear corepressor N-CoR to the TR, resulting in the repression of transcription. More recently, these authors have used the same approach with cellular and biochemical models to demonstrate that the anti-thyroid drugs methimazole and propylthiouracil significantly suppressed transcriptional activities mediated by TR. These compounds inhibit the action of T3 by enhancing the recruitment of transcriptional corepressors and/or the dissociation of coactivators [71]. Moreover, Iwasaki et al. [72] showed that 4-OH-PCB was able to suppress TR/coactivator (SRC-1) complex-mediated transactivation, and that this mechanism could explain the suppression of TR-mediated transcription in the presence of OH-PCB in simian kidney cells.

Such disrupting mechanisms could have biological implications, since a significant proportion of TRs are in the unliganded state at physiological concentrations of THs (euthyroid state), depending on the tissues concerned. Thus, about half of the TRs are in the unliganded state in the liver [73], and therefore the level of expression of regulated genes could be the result of silencing by apo-TRs and the stimulation of transcription by holo-TRs [74]. Moreover, unliganded TRs are predominant during the early stages of development in amphibians as well as in mammals, where they are

thought to exert important developmental functions before the onset of thyroid activity. Recent data have suggested that the apo-receptor is physiologically important during the early stages of development by maintaining some genes repressed until their induction by THs [1].

An additional level of complexity in the impact of endocrine disruptors on TH mechanisms of action is related to the down-regulation of a number of genes by liganded TRs [75]. This is exemplified by the down-regulation of TSH and thyrotropin-releasing hormone gene transcription by T3, which is responsible for the negative feedback exerted by THs on the hypothalamic-pituitary-thyroid axis, and is mediated by the β-isoforms of the TR. In a puzzling way, transcriptional corepressors increase the transcription of these genes [76], whereas transcriptional coactivators may have a role in their T3-dependent repression [77]. Thyroid-disrupting compounds are potentially capable of positive or negative interference in each of these circumstances, but the biological effects in a given tissue will be the resultant of a large number of factors, including the concentration of intracellular T3, the number of TRs, the availability of transcriptional corepressors and coactivators, and the mode of regulation of relevant target genes. It is essential to explore such potential mechanisms of action of TDCs.

Other potential mechanisms of action may involve crosstalk with other nuclear receptors. One study has demonstrated that PCB-OH can partially dissociate the heterodimer RXR/TR from T3–responsive element, a mechanism that could be involved in the suppression of transcription induced by PCB [78]. No other study has investigated this potential target of TDCs. RXR was reported to be a ‘‘silent partner’’ in the context of its heterodimerization with TR, unable to bind its ligand, 9-cis-retinoic acid, but more recent studies suggest that this retinoid may mediate the

stimulation of transcription by the TR-RXR heterodimer [79]. Therefore, in the context of its heterodimerization with TRs, RXR might be a target of TDCs, but this point has not yet been experimentally documented. It has been shown that peroxisome proliferator-activated receptors (PPARs), which form active heterodimers with RXR, may inhibit the transcriptional activity of TRs by competing for RXR, and consequently disrupting TR-RXR heterodimers [80]. The transcriptional activators of the SRC family are also shared by TR and PPARs [81]. Therefore chemicals such as phthalates, which are recognized PPAR disruptors [82], might indirectly alter TR transcriptional activity. Constitutive androstane receptor (CAR) and pregnane X receptor (PXR), which are both members of the nuclear receptor family, are activated by a large panel of xenobiotics, including endocrine disruptors, and induce the expression of drug transporters and xenobiotic metabolizing enzymes, including UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs). Hepatic SULTs and UGTs are involved in eliminating THs: the inactive glucuronide and sulfate derivatives of THs are eliminated in the urine and bile. Furthermore, T4 sulfate is a considerably better substrate than T4 for inner ring deiodination by liver type-I deiodinase [83], producing reverse T3, a biologically inactive metabolite of T4. CAR was shown to mediate the induction of several isoforms of UGTs and SULTs involved in glucuronidation and sulfation of THs [84]. Accordingly, Qatanani et al. [85] showed that CAR was required for the phenobarbital-mediated decrease in serum T4 concentration and the concomitant increase in serum TSH levels. Accordingly, treatment with CAR agonists resulted in disruption of TH homeostasis and induction of thyroid follicular cell proliferation. Therefore, CAR/PXR activation by xenobiotics could indirectly result in disruption of TR activity.

Some chemicals can also disrupt the expression of TRs. Sugiyama et al. [86]

developed a stably-transfected luciferase reporter gene assay to study interference with the TRβ1-signaling pathway by chemicals. Three types of phthalates, n-butylbenzyl phthalate (BBP), di-n-butyl phthalate and dicyclohexylphthalates (DCHPs), were reported to exhibit T3-antagonistic activity, at concentrations ranging from 10^{-6} M to 10^{-5} M. The authors noticed that in the absence of TDCs and the presence of T3, the amount of endogenous TRβ transcripts increased 13.3 fold in X. laevis cells. Interestingly, this T3-dependent activation was significantly inhibited by phthalates. The percentages of inhibition were 32% for DBP, 42% for DCHP, and more than 50% for BBP. Such an effect was also observed when tails of X. laevis tadpole in culture were exposed to bisphenol A at the concentration of 10^{-7} M. BPA was shown to block T3-induced tail resorption in a concentration-dependent manner. Analysis by semi-quantitative RT-PCR demonstrated that BPA reduced TRα and TRβ mRNAs levels, and also moderately suppressed RXR gene expression [87]. As far as we are aware, all the existing studies of this disruption have been carried using tadpole models, which makes it difficult to extrapolate the physiological consequences that may occur in humans.

4. Conclusions

Thyroid hormones are strongly involved in vertebrate brain development, from early embryogenesis to subsequent prenatal and perinatal development in mammals. The disruption of TH signaling in the developing human brain, may alter the process of neural differentiation and potentially account for the observation that exposure to TDCs is linked to cognitive deficits in humans. From this point of view, TDCs are of major concern for public health issues.

Several epidemiological data have indicated that pollutant exposure might lead to impaired developmental processes, which could be associated with the homeostasis and action of TH. The mechanisms of action of TDCs are complex and, although TR-independent mechanisms have been elucidated, there is a lack of data about TR-related mechanisms. A few compounds are known to have a direct affinity for TRs, whereas others are able to activate receptor-dependent transcription of TH target genes by modulating upstream signaling without binding to the T3 binding site of TRs. TDCs could exert transcriptional effects by disrupting the recruitment/release of coactivators by TRs, by interfering with the expression of TR and their heterodimerization partner, or by interfering with the affinity between TR and TRE. All these potential targets need to be investigated further, as do their physiological consequences. It is clear that TR-disruption by chemicals may interfere with physiological mechanisms that are reported to be important for normal development in amphibians and mammals.

Furthermore, several parameters considerably complicate the assessment of TDCs. The multiplicity of targets and the demonstration that many targets can be disrupted at the same time make it difficult to interpret results. On top of that, TDCs can also interfere with cellular processes that affect several different hormone-signaling pathways. The crosstalk between signaling pathways of different nuclear receptors is something has must be seriously considered in the future. Finally, although the effects of TDC are often less potent than those of TH, it is also important to keep in mind that humans are continuously exposed to a multitude of pollutants which can act together, and lead to effects that are different from those of the individual pollutants [88].

Due to the lack of available data, current concerns about the possible involvement of TDCs in the increased incidence of developmental diseases remain hypothetical. This review highlights the critical need to develop quick and robust tools to identify TDCs, and their multiple mechanisms of action, taking into account all the potential targets in the effects on TH transcription A better understanding of the mechanisms underlying TR-related disruption may lead to changes in public policy and awareness, and make it possible to limit adverse outcomes for future generations.

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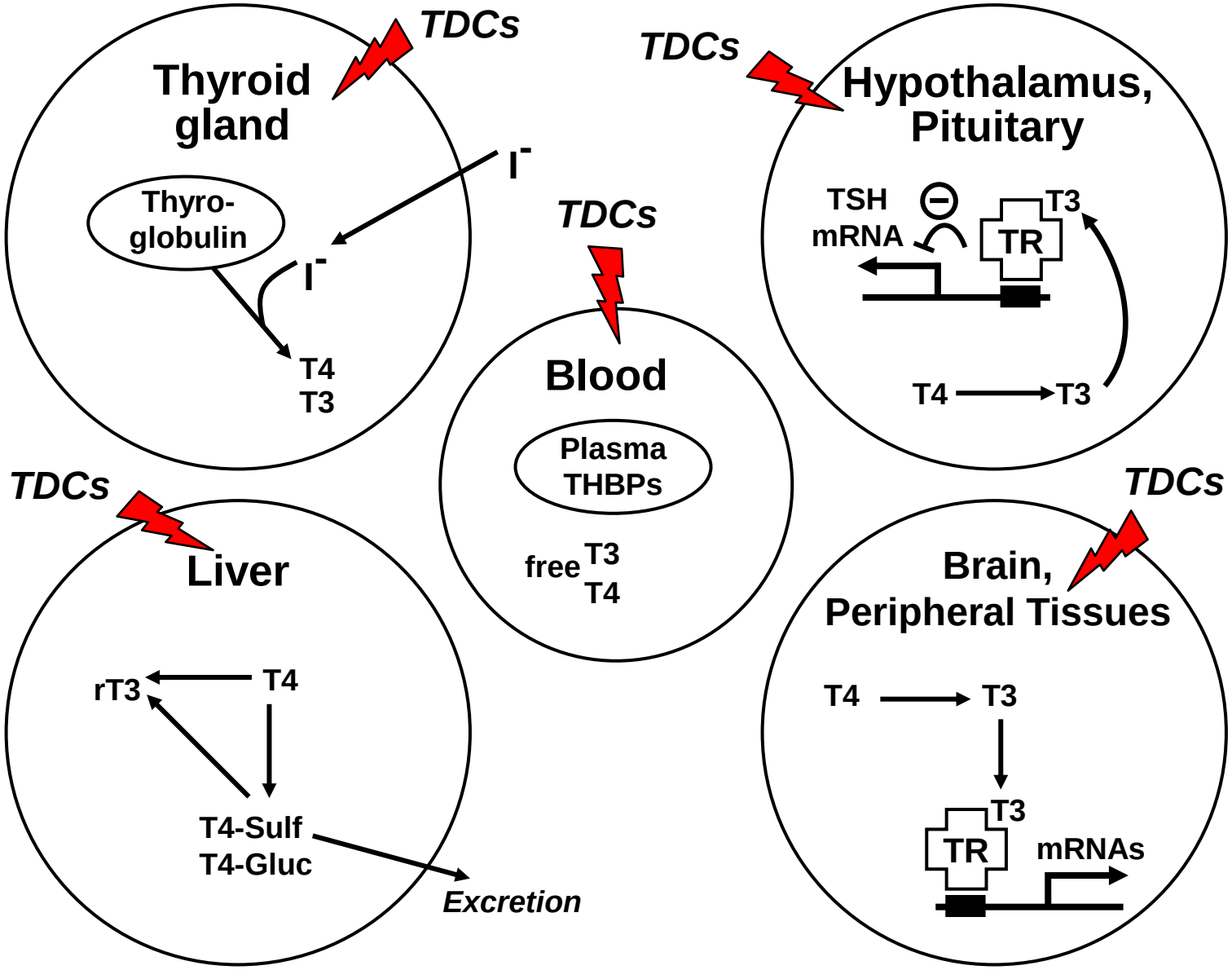
Graphical Abstract color



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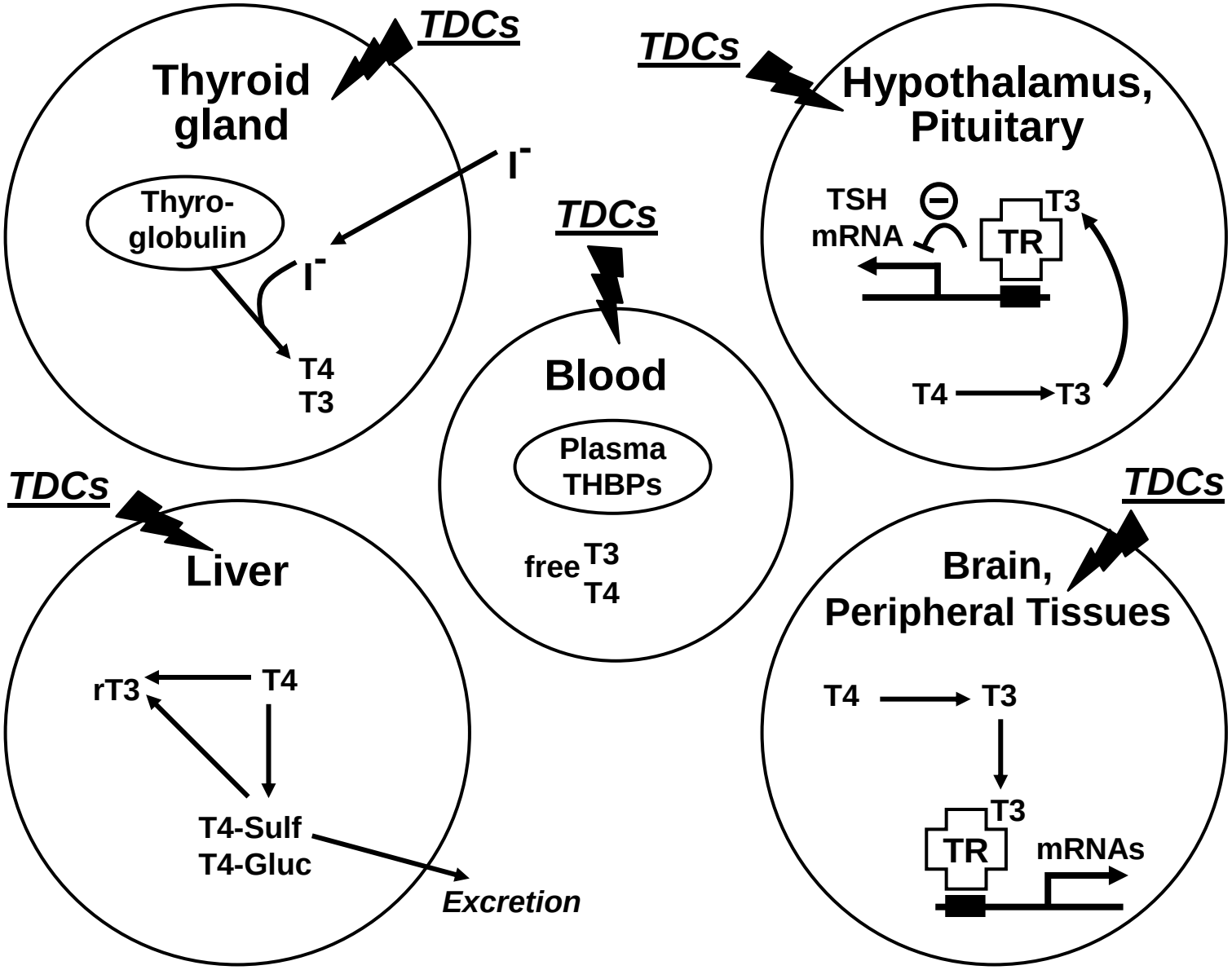


Table I: Cohort studies focusing on the association between mental and psychomotor defects and PCB perinatal exposure.

POPULATION OF CHILDREN	EFFECT RELATED TO PCB EXPOSURE	REFERENCES
N = 171 (7, 18, 30, 42 months) Germany	Pre- and post-natal exposure inhibits motor and mental development at all ages up to 42 months	[7]
9 years Netherlands	Higher prenatal PCB levels and lactational exposure were associated with longer and more variable response times, and lower psychomotor scores	[8]
N = 148 (11 years) USA (Michigan)	Prenatal PCB exposure was associated with greater impulsiveness, poorer concentration, and poorer verbal, pictorial, and auditory working memory. Postnatal exposure had no influence.	[9]
N = 788 USA (Massachusetts)	Association between low-level prenatal PCB exposures and poor attention in early infancy.	[10]
N = 232 (2 week, 18 months) Germany	No association between perinatal PCB exposure and mental and motor development defects	[11-12]
N = 1134 children and mother Slovakia	Maternal dioxin-like PCBs were significantly associated with lower scores on both the psychomotor and mental development indices. Anti-estrogenic and non-dioxin-like PCBs did not show any statistically significant association	[13]

Table II: Cohort studies that focus on the correlation between PCB exposure and TH levels. FT4: free T4, FT3: free T3, TT3: total T3, TPOab: anti-thyroperoxydase antibody, TBG: thyroid binding globulin; ND: no data.

POPULATION	PCB CONCENTRATIONS	CORRELATION	REFERENCES
149 pregnant women Quebec	0.3 µg/L blood	Negative: TT3 No: TSH, FT4, umbilical cord TT3	[17]
438 adult men Fish consumers USA (Great Lakes)	806 ng/g lipids in plasma 204 ng/g control (no fish consumption)	Negative: TSH, FT4 et TT3	[18]
232 adults Slovakia	< 530 ng/g lipids in plasma	Positive after high exposures: FT4, thyroid gland volume, TPOab	[19]
1307 adults Slovakia	0.53-2 µg/g lipids in plasma		
1123 adults Slovakia	2-101 µg/g lipids in plasma		
232 children 2 weeks, 18 months Germany	19.3 pg/g lipids in plasma	No: TSH, TT4, FT4, TT4, FT3	[12]
232 adolescents Canada (Quebec)	Persitent PCB: 0.32 ppb Non persistent PCB: 0.23 ppb in plasma	Negative: FT4 Positive: TSH	[20]
120 pregnant women USA (California)	0.89 µg/L (PCB-153) and 316 pg/g PCB-OH wet weight in plasma	Positive: TT3 No: TSH, FT4, TBG	[21]
95 umbilical cords	0.2 µg/L PCB-153 and 246 pg/g PCB-OH	Negative: TBG No: TT3, TSH, FT4	
130 children, 7 months	0.45 % g/L PCB-153	No: TBG, TT3, FT4, TSH	
38 adults	PCB 153: 0.42 ng/mL PCB 138: 0.31 ng/mL PCB 180: 0.36 ng/mL	Positive: FT4 No: TSH	[22]

Figure 1 color

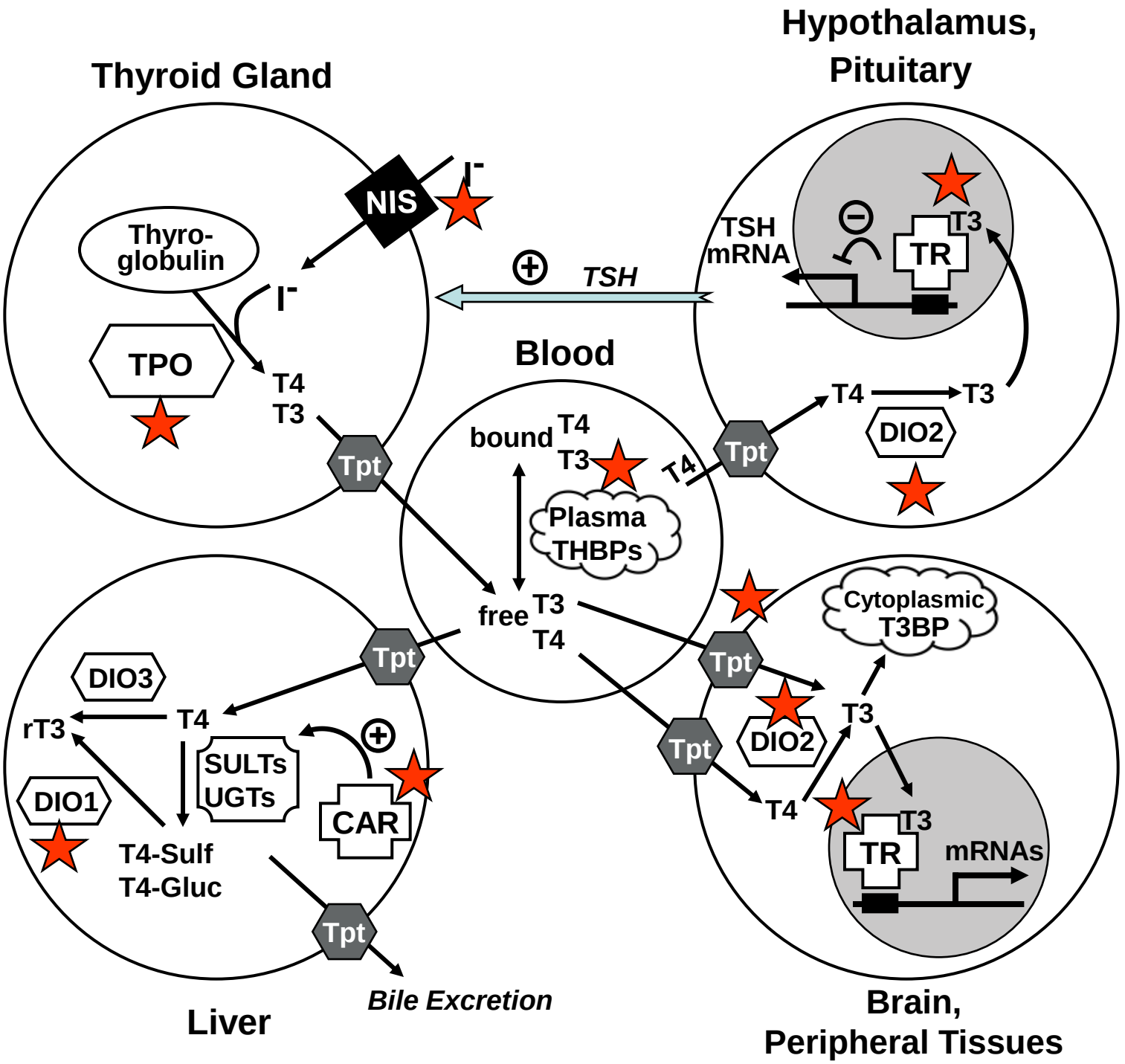


Figure 1 greyscale

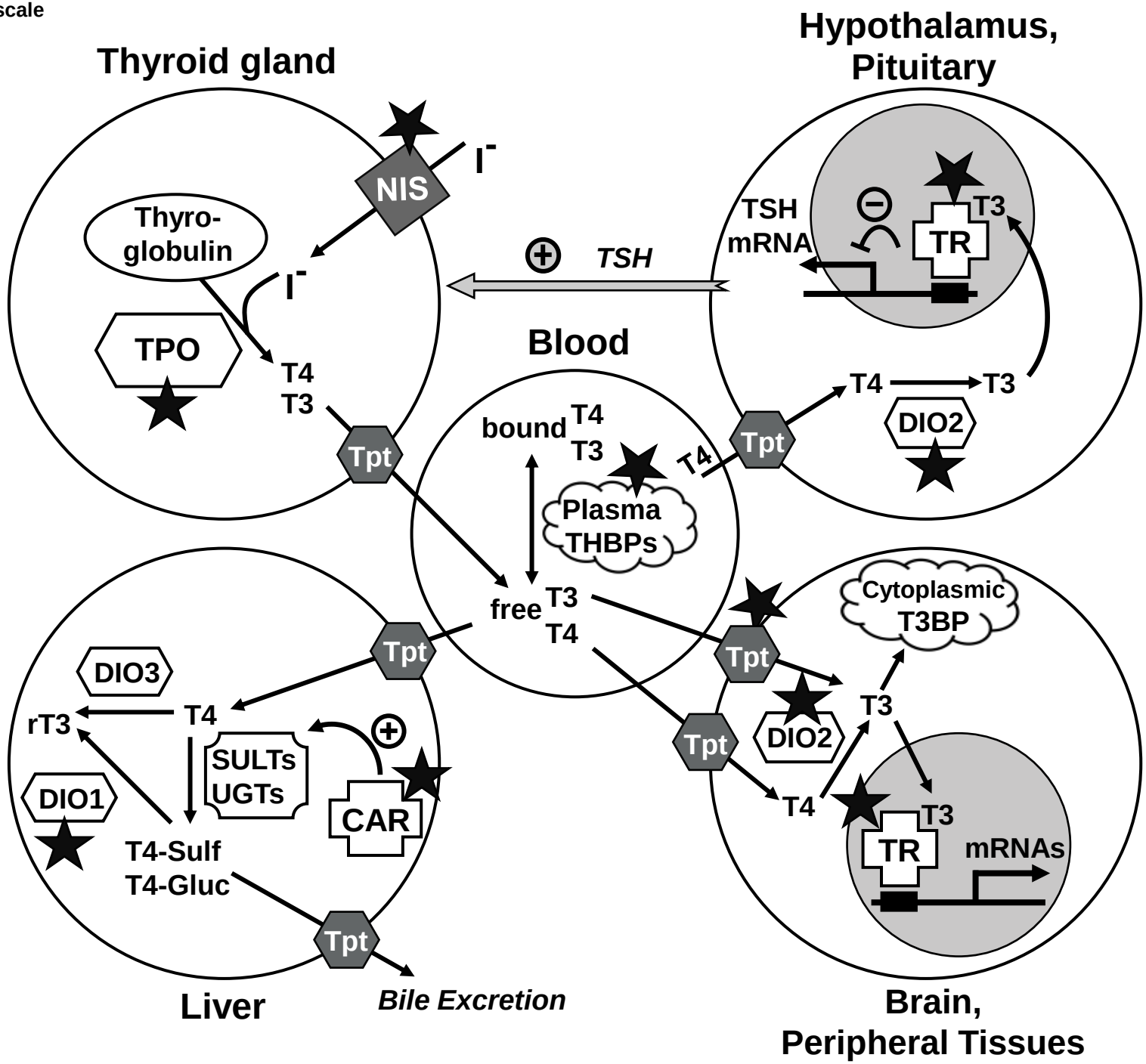
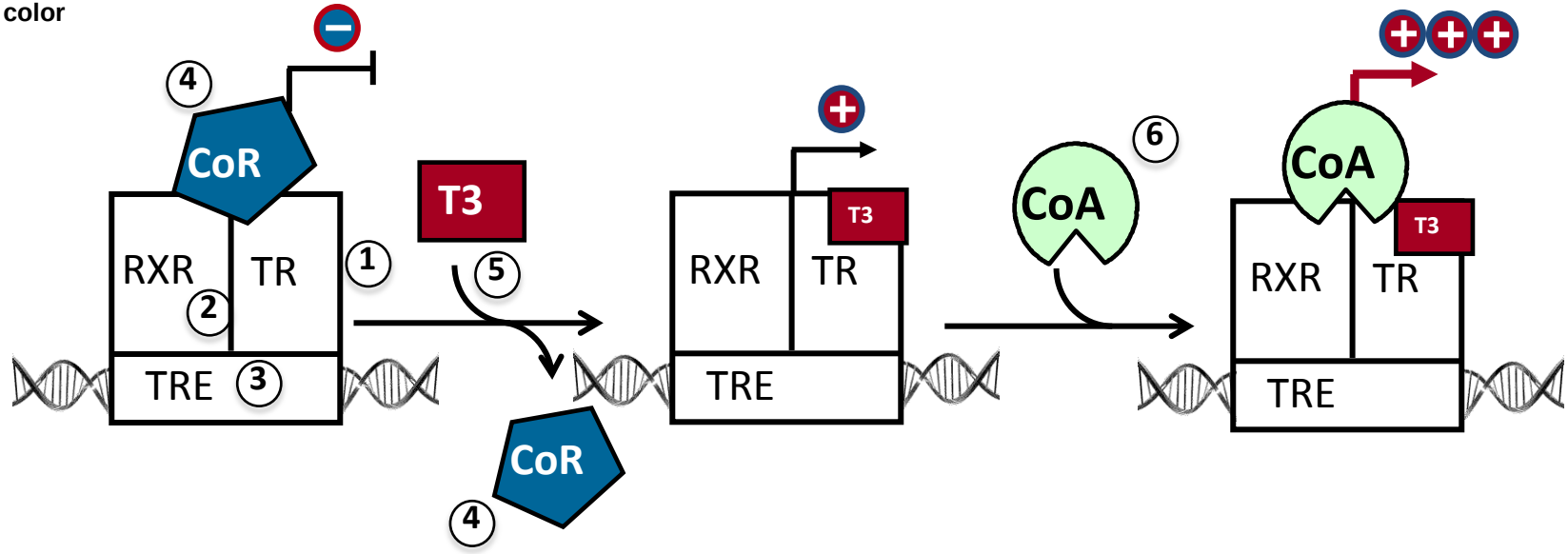


Figure 2 color



Endpoints	Chemicals	References
1. Expression of TR	Phthalates Bisphenol A	[86] [87]
2/ TR/RXR heterodimerization	No data	[80]
3/ Interaction of TRE with RXR/TR	OH-PCB	[78]
4/ Recruitment/release of corepressors~~,~~	Bisphenol A Anti-thyroid drugs NH-3	[70] [71] [69]
5/ T3 binding to TR	OH-PBDE Pentabromophenol Phthalates	[59-60] [60] [67]
6/ Recruitment of coactivators: transcriptional activation	OH-PCB	[72]

Figure 2 greyscale

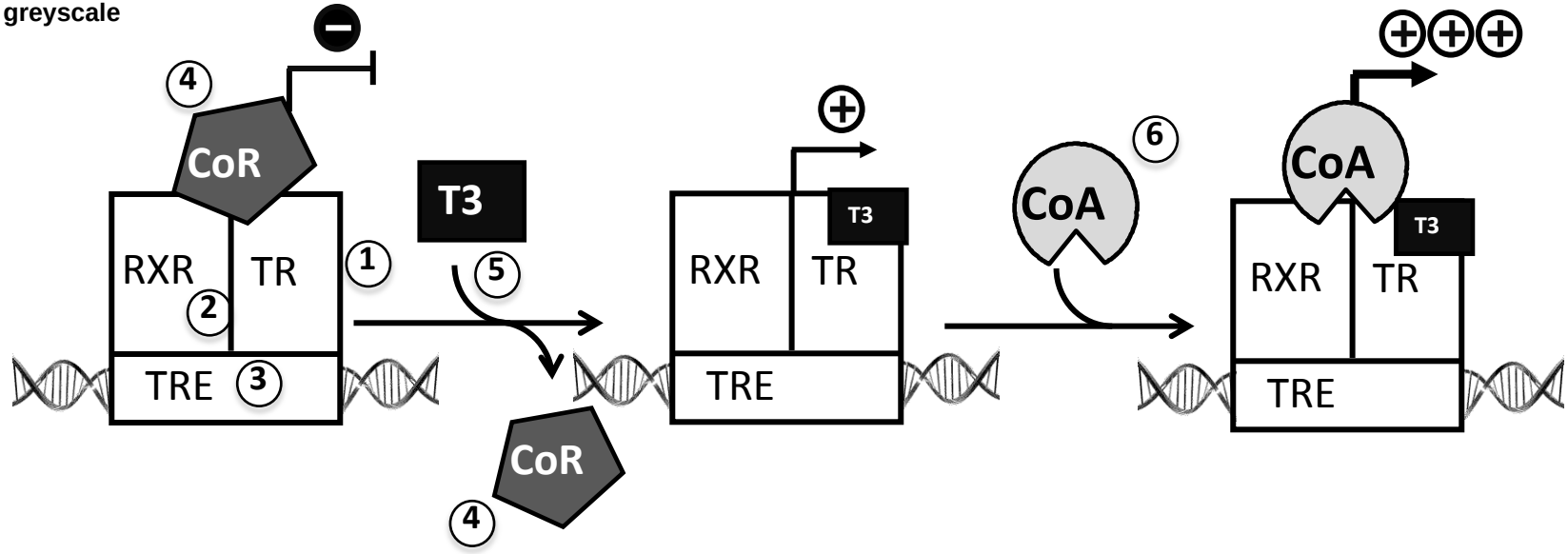


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FIGURE LEGEND

Figure 1: A schematic view of the thyroid hormone regulatoty network and thyroid disruption endpoints. Cytoplasmic T3BP: cytoplasmic T3-binding protein; DIO1, 2, 3: deiodinases type 1, 2, 3; NIS: sodium iodide symporter; Plasma THBPs: plasma thyroid hormone-binding proteins; rT3: reverse-T3 (inactive); SULT: sulfotransferase; T4-Gluc: T4 glucuronide (inactive); T4-Sulf: T4-Sulfate (inactive); TPO: thyroperoxydase; Tpt: membrane transporter; TR: thyroid hormone receptor; TSH: thyrotropin; UGT, glucuronosyltransferase; ★= thyroid disruption endpoints.

Figure 2: Regulation of transcription by the thyroid hormone receptor (TR). In the absence of T3, the heterodimer TR-RXR, bound to a T3-responsive element (TRE) on DNA, recruits corepressors (CoR) and represses basal transcription. T3 binding to TR induces the release of CoR, and restores the basal transcription level. Coactivators (CoA) can then be recruited and stimulate transcription. Table inset: Environmental chemicals may target various levels of the TH-related transcriptional process.