Impact of endocrine disrupting chemicals on neurodevelopment: the need for better testing strategies for endocrine disruption-induced developmental neurotoxicity

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ABSTRACT

**Introduction:** Brain development is highly dependent on hormonal regulation. Exposure to chemicals disrupting endocrine signaling has been associated with neurodevelopmental impairment. This raises concern about exposure to the suspected hundreds of endocrine disruptors, and has resulted in efforts to improve regulation of these chemicals. Yet, the causal links between endocrine disruption and developmental neurotoxicity, which would be required for regulatory action, are still largely missing.

**Areas covered:** In this review, we illustrate the importance of two endocrine systems, thyroid hormone and retinoic acid pathways, for neurodevelopment. We place special emphasis on TH and RA synthesis, metabolism, and how endocrine disrupting chemicals known or suspected to affect these systems are associated with developmental neurotoxicity.

**Expert opinion:** While it is clear that neurodevelopment is dependent on proper hormonal functioning, and evidence is increasing for developmental neurotoxicity induced by endocrine disrupting chemicals, this is not grasped by current chemical testing. Thus, there is an urgent need to develop test methods detecting endocrine disruption in the context of neurodevelopment. Key to this development is further mechanistic insights on the involvement of endocrine signaling in neurodevelopment as well as increased support to develop and validate new test methods for the regulatory context.

1. Introduction

Hormones play a fundamental role during fetal brain development influencing key processes such as proliferation, apoptosis, differentiation, migration, and myelination [1–5]. Thus, the developing brain is rendered vulnerable to environmental stimuli that can disturb the hormonal milieu. This raises concerns about health consequences of developmental exposures to a group of chemicals known as endocrine disrupting chemicals (EDCs), which are capable of interacting with various hormonal pathways at different levels. EDCs are exogenous substances or mixtures capable of interacting with the endocrine system and leading to adverse effects in intact organisms, their progeny or (sub) populations [6].

EDCs have been shown to be present in biological fluids of pregnant women [7,8] and there is emerging epidemiological evidence suggesting associations between exposure to EDCs and negative neurodevelopmental outcomes in children. For instance, exposure to EDCs has been associated with disorders indicative of developmental neurotoxicity (DNT) such as autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and intellectual disabilities (for a comprehensive review see [9]). Briefly, Bisphenol A (BPA), phthalates, and perfluoroalkyl substances (PFAS), have been associated with ASD [10] and social behavior disturbances which may be significant indicators of ASD [11–17]. Likewise, links between ADHD-related neurobehavioral traits and BPA [18], phthalates [19,20] or organic persistent pollutants [21] have been established. Intellectual disabilities have also been reported to be associated with EDC exposure, whereby intellectual quotient (IQ) was used in many studies as a measure of intellectual capacity. Lower IQ scores have been correlated to some metabolites of phthalates [22], bisphenol F (BPF) [23] and different mixtures of EDCs [24,25] in different settings. BPA has been shown to have detrimental effects on executive functions [26], working memory [12] and language development [27]. Moreover, behavioral disturbances [28,29] and academic achievement [30] have also been shown to be associated with EDC exposure.

Although many epidemiological studies have shown associations between neurodevelopmental outcomes and prenatal exposure to EDCs, at present the epidemiological literature remains inconclusive due to the heterogeneity and inherent limitations of such studies. However, the epidemiological evidence is supported by data obtained from animal studies showing that in utero/peri- andnatal exposure to EDCs can lead to effects on behavior, memory, and motor activity (reviewed in [31–34]).

In Europe, EDCs are currently identified using hazard-based criteria in the context of EU Regulations (EC) No. 1107/2009 Plant Protection Products Regulation (PPPR) and (EU) No. 528/
2012 Biocidal Products Regulation (BPR). The requirements for determining whether a substance meets the definition specified in the EDC criteria for PPPR and BPR include evidence that the substance has endocrine activity and that there is a biologically plausible link between the endocrine mode of action and the adverse effect. However, the few available in vivo DNT tests do not address effects induced through endocrine modes of action, which highlights the importance of, scientifically and methodologically, bridging the gap between EDCs and DNT from a regulatory perspective.

In this review, we aim at illustrating the importance of hormonal functioning for neurodevelopment, and thus the need to consider endocrine disruption in the field of DNT. There are a number of endocrine pathways involved in neurodevelopment. For example, sex steroids, the arguably most studied hormones in the context of endocrine disruption, are involved in the development of sexual dimorphism of the brain and mediate neurodevelopmental processes such as neuronal apoptosis, neurite growth, differentiation, and activity of dopaminergic neurons, oligodendrocyte maturation, myelination, synaptogenesis, and synaptic plasticity [35,36]. This review is focused on two other examples, the thyroid hormone and retinoic acid systems, which we use to describe the molecular mechanisms linking hormonal signaling with neurodevelopment. Furthermore, we present evidence clearly linking their disruption by EDCs with DNT outcomes, and provide an overview of current efforts to develop models capable of detecting ED-induced DNT.

2. Thyroid hormone disruption and developmental neurotoxicity

Thyroid hormone (TH) plays a crucial role in prenatal development, and many adverse consequences arising from thyroid diseases during pregnancy are well described in the literature. Fetal hypothyroidism can, in the most extreme cases lead to cretinism, which manifests, among others, with intellectual disability [37]. Also maternal hypothyroidism is associated with lower IQ scores, as well as with ASD and ADHD in different populations [38–40]. Brain morphology has also been demonstrated to be affected by maternal hypothyroidism [2,41]. Correspondingly, the link between maternal hyperthyroidism and cortical gray matter alterations has been established [2,41]. At the cellular level, TH is involved in neuronal and oligodendrocyte differentiation and maturation [42,43].

2.1. TH synthesis, signaling, and metabolism

When stimulated by thyroid stimulating hormone (TSH) secreted by the pituitary gland, the thyroid gland produces the thyroid hormones thyroxine (T4), and triiodothyronine (T3) [44] (Figure 1). Acquired from seafood, dairy, and fortified food items such as salt, iodine is essential for TH synthesis. Iodine is absorbed in the stomach and small intestine and stored in iodine concentrating tissues such as the thyroid gland, the salivary gland, lactating breast, intestine, and stomach [45]. Once in the bloodstream, iodine is transported into storing tissues by the action of the sodium/iodide symporter (NIS), whose transcription and insertion in cellular membranes is regulated by TSH [46,47] (Figure 1). Congenital iodine transport defects associated with hypothyroidism and goiter have been linked to mutations in SLC5A5, which encodes for NIS [48]. Iodine in thyroid follicular cells is transported through the cellular apical membrane toward the follicular lumen by pendrin, a member of the SLC26A family [49,50]. Upon uptake by the thyroid, iodine is oxidized and added to tyrosine residues in thyroglobulin by thyroid peroxidase (TPO), resulting in the formation of monoiodothyronine (MIT), diiodothyronine (DIT), T3, reverse T3 (an isomer of triiodothyronine), and T4; although T3 is mainly produced by T4 deiodination (reviewed in [51]). During gestation, maternal iodine intake and placental transport are the main contributors to the fetal iodine supply [52].

T3 exerts most of its functions by binding to the Thyroid hormone receptor (THR), which can then dimerize and form homodimers or heterodimers with retinoic X receptors (RXRs). The dimers then bind to thyroid hormone response elements (TRE) in the DNA and initiate gene transcription of target genes [4]. On the other hand, low intracellular T3 levels induce the recruitment of nuclear corepressors leading to repression of target genes. THRα are divided into two subtypes, encoded by two genes, named thyroid hormone receptor alpha (THRα) and thyroid hormone receptor beta (THRβ) with the main differences at the level of amino acids present in their DNA binding domain (reviewed in [53]). In the developing rat brain, 60% of THRα are THRα, while THRβ appears in the brain at late developmental stages and its expression is more limited to specific areas (e.g. hypothalamus, pituitary, retina, and cochlea) [54]. It is, however, important to note that limitations regarding the specificity of antibodies for THRα and THRβ have been reported [55] posing a problem for the identification of these receptors in the developing brain. In humans, germ-line mutations in the THRα lead to a condition similar to congenital hypothyroidism [56]. On the other hand, germ-line mutations in THRβ produce a condition characterized by resistance to TH with elevated T3 levels and varied tissue sensitivity to the hormone. In this case, manifestations include a myriad of symptoms typical of both hypo- and hyperthyroidism (reviewed in [57]). TH can also modulate important
cellular responses via non-canonical pathways such as the PKA pathway, modulation of the expression of epidermal growth factor (EGF) and fibroblast growth factor (FGF), and the interaction between T4 and integrin αvβ3 [58]. Likewise, T3 has been reported to trigger generation of Ca2+, NO, inositol triphosphate and cAMP within minutes after its administration, suggesting that these effects are not mediated by transcriptional changes [59].

TH production is regulated by negative feedback in the hypothalamic-pituitary-thyroid (HPT) axis (Figure 1). Circulating TH levels are sensed in the hypothalamus and pituitary gland, which in turn decrease the production of TSH by the anterior pituitary and/or of thyrotropin-releasing hormone (TRH), produced in the hypothalamus [4]. Due to its hydrophobicity, TH binds to plasmatic proteins in the circulation (thyroxine-binding protein, transthyretin, and albumin), which facilitate hormonal transit. At the cellular level, T4 and T3 are taken up by the cells via monocarboxylate transporters (MCT), which prefer T3, l-amino acid transporters (LAT) which transports both T3 and T4, and organic anion transporters polypeptides (OATP) which transport mainly T4 and reverse T3 [60]. In the brain, MCT8 and MCT10 are the main transporters, and a mutation in MCT8 is linked to the Allan-Herndon-Dudley syndrome (AHDS), characterized by intellectual impairment and a myriad of neurological symptoms [61]. In the fetus, the thyroid gland develops from weeks 5 to 6 of gestation, but fetal TH synthesis starts later on (weeks 14–16 of gestation) [62,63]. Fetal hypothalamic–pituitary–thyroid axis (HPT) starts to function at the beginning of the third trimester [63] and TH reaches adult levels after birth. Thus, fetal TH levels in early gestation are dependent on maternal transfer regulated by placental transporters, deiodinases D2 and D3, and placental synthesis and secretion of transthyretin and albumin [64,65].

2.2. TH signaling and neurodevelopment

In the developing brain, TH is involved in cell proliferation, neurogenesis myelination and proliferation of oligodendrocyte precursors, glia-neuron communication, and the formation of Bergmann’s glia [4]. T4 is actively transported across the brain-blood barrier (BBB), whereas BBB is mostly impermeable to T3 (reviewed in [66]). T4 entering the brain after moving across the endothelial cells, is taken up by astrocytes by an OATP transporter (Oatp1c1), where it is deiodinated by D2 producing T3, which is then able to exit the astrocytes via MCT8 and be taken up by neurons and oligodendrocytes via MCT8 [67] (Figure 1). Once inside the cell, 5’-deiodinases will remove iodide from TH depending on the current cellular needs. Three types of deiodinase enzymes have been described, D1 and D2 are responsible for the conversion of T4 to T3, D1 and D3 convert T4 to reverse T3, and D1, D2 and D3 convert T3 and reverse T3 to T2. The main deiodinases found in the brain are D2 and D3, in fact most of the T3 in the brain comes from D2 expressed in astrocytes, tanycytes and oligodendrocyte progenitor cells.
The expression of TH transporters MCT8, MCT10, OATP1A2, OATP4A1, LAT1 and LAT2 have been reported in placenta [69,70]. At the cellular level, TH regulates neural stem cell fate, oligodendrocyte differentiation, and differentiation of dopaminergic neurons. Oligodendrocyte differentiation is dependent on T3 in part due to T3 mediated secretion of neurotrophins by other cell types (reviewed in [55,66]).

2.3. Chemical exposures causing disruption in the thyroid hormone signaling pathway leading to DNT

EDCs can interfere with TH signaling by interfering with TH synthesis and circulating levels, blocking TPO enzymatic activity or iodine transport, or disrupting nuclear receptor signaling [71] (Figure 1). For instance, perchlorate (ClO4−) compounds interfere with NIS, inhibiting iodine transport due to the increased affinity ClO4− has for NIS in comparison with iodide [72]. ClO4− has been used in detonators, pyrotechnics, explosives, airbags, rocket fuels, PVC production, fertilizers, and it is also produced by degradation of hypochlorite (reviewed in [73,74]). Taylor P.N. et al. investigated the correlation between urinary ClO4− in the first trimester of pregnancy in mothers with hypothyroidism and supplementation with levothyroxine, finding increased odds for lower IQs in their children at 3 years of age [75]. This association is supported by experimental data where gestational exposure to ClO4− decreased T4 levels and reduced hippocampal synaptic transmission in rats [76].

Another group of chemicals affecting TH homeostasis are pesticides from the organochlorine, organophosphate, carbamate, pyrethroid and neonicotinoid families. A recent review by Leemans et al. reported that organochlorine pesticides such as DDT and hexachlorobenzene have been associated with decreased total and free T3, decreased free T4 and increased TSH in multiple mother and child cohorts [77]. Likewise, decreased levels of T4 and altered intellectual development have been associated with exposure to organophosphates [77]. These associations are supported by experimental in vivo data where pesticides have been found to affect TH levels, decrease circulating transthyretin, affect the hepatic metabolism of T4, induce histological changes in the thyroid and reduce brain weight. For a comprehensive review the reader is referred to [77].

Polychlorinated biphenyls (PCBs) are persistent organic pollutants which were used for various applications and were present in transformers, electrical capacitors, hydraulic fluids and paints. Although they are no longer made, PCBs can still be found as part of products produced before the ban [78]. PCBs act on THR as agonists or antagonists and can affect TH levels in humans and experimentally in vivo [78,79]. In epidemiological studies, PCBs have been associated with increased risk of hyperactivity and attention deficit among others (reviewed in [80]). Exposure to a PCB mixture induced changes in serum TH concentration in dams together with altered cell cycle exit of neuronal progenitors and delayed radial glia migration in the cortex of the developmentally exposed pups [79].

Also chemicals used in plastic production, phthalates and bisphenols, are suspected to interfere with TH signaling.

Phthalates have been shown to affect TH signaling in different ways, including altering circulating TH levels, increasing thyroglobulin expression, upregulating D1 and D2, decreasing transthyretin levels and decreasing the expression of THRα and THRβ (reviewed in [81]). A recent study by Derakhshan et al. showed that exposure to phthalates was associated with lower circulating levels of TH including free T4 and free T3 in pregnant women [8]. Furthermore, prenatal exposure to phthalates has been associated with disturbances in child motor development and lower scores in mental and psychomotor indices assessed with the Bayley scales [82,83]. In a recent study, Bornehag et al. found associations between first-trimester maternal urinary levels of dibutyl phthalate (DBP) and butyl benzyl phthalate (BBP), and language delay in children 2.5 to 3 years old [84]. Finally, BPA, used, e.g. as starting material for most epoxy resins, and its analogues BPF and BPS can bind to THR and act as either agonist or antagonist. BPA has been associated with altered serum TH levels in pregnant women [85,86] as well as with neurodevelopmental impacts in epidemiological and experimental data (reviewed in [87]). These studies show, on occasion, contrasting results, in part explained by differences in species, sex, administered doses and tests used for evaluation of neurodevelopment.

In conclusion, there is evidence from an increasing number of studies for TH disruption by industrial chemicals on one hand, and associations between exposure to these chemicals and neurodevelopmental impacts on the other hand. Yet, studies clearly linking DNT and TH disruption in the same population or experimental setting, are very rare.

3. Involvement of retinoids in brain development

Retinoids are essential for both embryonic and adult growth. Retinoic acid (RA), a metabolite of vitamin A, is involved in the development of the heart, limbs, nervous system, and through the regulation of Homeobox (HOX) genes, RA directs anterior-posterior patterning in the embryo [88]. At the cellular level, RA has been widely demonstrated to induce differentiation of neurons and glia, and to increase the number and the length of neurites [89].

3.1. RA synthesis, signaling, and metabolism

Retinoids are acquired from the diet and are precursors for at least two important metabolites, all-trans-retinoic acid, and 9-cis-retinoic acid. In order to become biologically active, retinol has to be absorbed in the gut, metabolized and transported to various tissues. The transport of retinoids into the cells is facilitated by retinoid binding proteins (RBP) that bind to the STRA6 receptor (stimulated by retinoic acid 6), enabling the hydrophobic retinoids to enter the cells [90] (Figure 2). The catabolism of retinol to retinoic acid consists in a two-step oxidation. Initially, retinol is converted to retinaldehyde by two enzyme families, retinol dehydrogenases (Rdh10) and alcohol dehydrogenases (ADHs) [91]. Retinaldehyde can be converted back to retinol by dehydrogenase/reductase enzymes (Dhr), regulating the amount of substrate available for retinoic acid synthesis [92]. The second and final step in this synthesis is the oxidation of retinaldehyde to retinoic acid facilitated by
During activation, the 4-oxo-retinoic acid is metabolized RA into the less bioactive 4-oxo-retinoic acid. Retinoic acid binding proteins (CRABPs) transport RA into the nucleus where RA binds to corresponding response elements. Acrylamide has been shown to downregulate the expression of Crabp2 and Rbp7, likewise VPA downregulates Cyp26a1 and affects Aldh1a2 altering RA signaling.

Once retinoic acid is synthesized, it is transferred to the nucleus by cellular retinoic acid binding proteins (CRABPs), where it acts as a transcriptional activating ligand by binding to the nuclear retinoic acid receptors (RARs) and RXRs (Figure 2). RARs recognize both all-trans-retinoid acid and 9-cis-retinoid acid, while RXRs only recognize 9-cis-retinoid acid. Upon binding, the receptors form a heterodimer complex, activating gene transcription by binding to retinoic acid response elements (RARE) in the genome [97]. RXR forms heterodimers with other hormonal nuclear receptors such as the thyroid hormone receptors (TRs), vitamin D receptors, peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXR) [98,99].

3.2. RA signaling and neurodevelopment

RARs and RXRs have been found to be expressed from early developmental stages in zebrafish and xenopus (Reviewed by [100]). In mammals, a study in mouse totipotent-like cells resembling the blastomere of 2-cell embryos showed expression of Rars and Rxr and activity in response to RA, implying activation of the RA pathway at this developmental stage [3]. During gastrulation, RA is restricted to the posterior region by the presence of CYP26A1 and CYP26C1 in the anterior region. Once the neural plate is formed, RALDH2 is transiently expressed in the rostral forebrain leading to activation of RA in this region and regulation of cellular signals such as Wnts, fibroblast growth factor 8 (FGF8), and sonic hedgehog (SHH), which are needed for the development of telencephalic, diencephalic and optic vesicles [100–102]. As the embryo continues developing, RA-dependent signaling is present in the primitive streak, primitive node and in the mesoderm, while at the same time CYP26A1 and CYP26C1 maintain anterior embryonic regions free from RA [103–108]. At this stage the expression of RA and RXR receptors centers on the neural ectoderm and a differential expression of RAR subtypes is observed with a strong downregulation of Rarg in the neural plate and mesoderm. At this point, the developing brain subdivides into three regions: the forebrain (which will become the cerebral cortex, basal ganglia and hippocampus), the midbrain, and the hindbrain (which will become the cerebellum, pons and medulla). RA concentrates in the hindbrain, where it is essential for the organization of segmented regions called rhombomeres. As embryonic development progresses, RA becomes indispensable for the development of anterior structures and forebrain growth [100,109], with both RALDH2 and RALDH3 as the main RA producing enzymes in the frontonasal region. During late brain development, Rara is expressed almost exclusively in regions derived from the hindbrain (medulla oblongata, pons and cerebellum), the corpus striatum and corpus pallidum, whereas Rarb expression is found in the medulla oblongata, pons, cerebellum, choroid plexuses, and the developing meninges [110,111].

The balance of bioavailable retinoic acid is primarily driven by Rhd, Raldh and CYP26 enzymes, by regulating the synthesis
and degradation of retinoic acid. The expression of these enzymes is tissue-dependent and highly regulated during neuronal development. Loss of Rdh10 activity has been shown to cause a deficiency of bioavailable retinoic acid and leads to craniofacial and limb developmental defects in mice [112,113]. Similarly, loss of Raldh2 and Raldh3 function in mice leads to cardiac and forelimb development deficiency [114,115]. Disruption of the CYP26A1 and CYP26B1 enzymes in mice, leading to an increase of retinoic acid in the cell, causes abnormalities in the hindbrain patterning, proximal limb patterning, resembling the teratogenic effects of RA [116,117].

Although RA is essential for neurogenesis in both embryo and adult, this morphogen was demonstrated to be teratogenic. Thus, both a deficiency and an excess of RA can lead to negative effects on the neurodevelopmental process [88,118]. For example, the removal of vitamin A from maternal diet in animals caused problems such as lack of eyeballs in pig offspring, hydrocephalus, spina bifida and microphthalmia [89,119–122]. Furthermore, the lack of this vitamin during in utero development led to abnormalities at different levels in quails, rats, chick and mouse embryos [1]: in the development of the caudal hindbrain [2], in the formation of the spinal cord [3], and by causing the death of the neural crest cells [89,123–127]. In addition, Bao et al. (2012) reported an increase in the risk of developing schizophrenia or other schizophrenia spectrum disorders after retinol deficiency during the second trimester of pregnancy [118,128–131].

On the contrary, excess of RA and vitamin A have been demonstrated to be teratogenic [89,132–135] producing defects at the level of the eye, the ear, the spinal cord and the hindbrain [89,123,136–140]. 13-cis RA causes on the one hand hydrocephaly and malformations in all the hindbrain structures especially in the precerellar nuclei [89] leading to motor and sensory developmental delays and to severe mental retardation [89,141,142]. On the other hand, it can also lead to microcephaly or a decrease in size of specific forebrain regions, together with occasional cortical heterotopias [89]. It is also noteworthy that children embryonically exposed to 13-cis RA and born without major malformations often exhibit cognitive impairments, such as reduced mental ability [89,141–143]. In addition, human males seem to be more vulnerable to cognitive impairments than females, although the reason for this difference has not been elucidated yet [142].

3.3. Chemical exposures correlated with disruption in the retinoic signaling pathway leading to DNT

The inhibition of RA signaling has been proposed as one of the mechanisms underlying the changes evidenced in the fetal alcohol spectrum disorder (FASD). This link has been made based on the role of ADH and CYP450 enzymes in both RA and alcohol metabolism [144]. FASD is characterized by anatomical and neurodevelopmental abnormalities and, in the most severe cases, microcephaly and growth restriction [144]. Animal data supports these claims as some teratogenic effects induced by ethanol have been rescued by supplementation with retinoic acid [145–147].

To the best of our knowledge, the effect of anthropogenic chemicals on RA signaling in correlation to DNT has not been addressed epidemiologically or in vivo experiments. However, in vitro, chemicals known to produce DNT have been reported to alter RA homeostasis (Figure 2). For example, exposure to valproic acid (VPA), an anticonvulsant commonly used for epilepsy treatment [148], has shown to cause neural tube malformation and significantly downregulate Cyp26a1 gene expression in mouse embryos and pluripotent mouse embryonal carcinoma cells (P19), resulting in altered retinoic acid signaling [149–152]. Connection between VPA exposure and RA signaling has also been found in an in vitro mouse gastrulation model of P19CS stem cell derived embryoid bodies. In this model, several genes involved in retinoic acid metabolism were altered (Aldh1a2 and Cyp26a1) and axial patterning and elongation were inhibited, moreover these effects were partially rescued by the use of a RA antagonist [153]. Acrylamide, is an organic compound used to produce polyacrylamide widely used in petroleum applications, in water and wastewater treatment, as a soil conditioner [154]. Acrylamide has also been used in cosmetics and textiles and it is formed when starch rich foods are cooked at high temperatures (reviewed in [155]). Maternal exposure to acrylamide has been associated with reduced fetal growth and head circumference, whereas in adults, it has been associated with hearing loss and mild cognitive decline [155]. Since other DNT effects have not been studied, acrylamide is a suspected DNT. In vitro, acrylamide has shown to alter neurodevelopmental differentiation and downregulate the expression of genes involved in RA signaling (e.g. Crabp2 and Rbp7) in differentiated SH-SYSY cells [156].

Altogether a clear link between chemically induced RA disruption and DNT has not been established. Although there is vast knowledge about the fundamental role of RA signaling during neurodevelopment, there is a lack of studies addressing RA disruption in relation to DNT.

4. Expert opinion

The two pathways reviewed here illustrate the dependence of neurodevelopment on proper hormonal functioning, and thus the link between endocrine disruption and developmental neurotoxicity. However, this link remains largely unaddressed in a correlative, let alone causative manner. Yet, a causative link between an endocrine mode of action and a DNT outcome would be the requirement to regulate EDCs according to the criteria currently used by the EU. Thus, DNT induced by ED is not appropriately covered in the current regulations on chemical testing. In fact, at present, the Organization for Economic Co-Operation and Development (OECD) does not have approved test guidelines (TGs) which can properly address ED induced DNT. The two OECD approved TGs addressing DNT in vivo, TG426 and TG443, are not required to be performed unless a compound shows evidence of neurotoxicity in repeated dose studies, or evidence of thyroid disrupting activity. The endpoints measured in TG426 and TG443 consist in gross anatomical evaluation of brain tissue, histopathological examination, and a very limited number neurobehavioral tests, with only optional evaluations of social and cognitive impairments, which are often seen with
exposures to EDCs. Therefore, ED-induced manifestations of developmental neurotoxicity are likely missed by the chemical assessment required by current regulations.

Furthermore, the in vivo rodent chemical testing has significant cost and time requirements, as well as translational issues, an approach which is unsustainable and unsuitable for the evaluation of thousands of chemicals for DNT potential [157,158]. As a result, there has been a great interest in developing New Approach Methods (NAMs) for DNT, which represent non-animal based approaches that can be used to provide information in the context of chemical hazard and risk assessment [159,160]. For DNT, efforts have been especially dedicated to the development of in vitro models which recapitulate key events during neurodevelopment, such as proliferation, migration, neural differentiation, neurite outgrowth, and neural network formation and function [157,161]. At present, these models are in different stages of development and still require international validation to be used for regulatory decision-making [161]. Ideally, the future toxicological testing strategy will shift toward Integrated Approaches to Testing and Assessment (IATA), implying integration of data from in vitro testing batteries with other streams of evidence to guide risk management decisions and the prioritization of chemicals for in vivo DNT testing [161].

To aid in the development of IATA for ED-induced DNT, the Adverse Outcome Pathway (AOP) concept has been introduced as a promising tool, as it represents an objective systematic approach and a practical framework for the organization and understanding of toxicological knowledge [162]. AOPs are constructed based on a sequence of causally linked and measurable Key Events (KEs), which connect a molecular initiating event (MIE) to an adverse outcome (AO) through different levels of biological organization [162]. Although AOPs contain an immense potential to integrate basic research with regulatory needs, AOPs for ED-induced DNT are difficult to develop at this time due to the lack of mechanistic knowledge related to endocrine mediated processes driving neurodevelopment, the importance of timing of such processes, and crosstalk between endocrine and other physiological systems. It is also worth mentioning that in the two cases exposed in this review (TH and RA) there is a good amount of knowledge available on their role in neurodevelopment; however, this is not the case for other hormonal pathways.

Building on these efforts, the EU, through the European Cluster to Improve Identification of Endocrine Disruptors (EURION: https://eurion-cluster.eu/), is currently supporting the development and validation of NAMs for the assessment of the effects of EDCs on neurodevelopment (ENDpoiNTs project) and also on TH disruption (ATHENA: http://athenaedctestmethods.net/; ERGO: https://ergo-project.eu/; and SCREENED: https://www.screensed-project.eu/ projects) [163–166]. Other efforts at further studying TH disruption outside the EURION cluster include the EC thyroid feasibility study which finished in 2019 with a report [167] and the Cefic-Lri – Long-Range Research Initiative. Likewise, efforts leading to the development of AOPs involving the endocrine system are currently taking place within and among these projects. Ultimately, it is expected that these efforts will help clarify the link between ED and DNT leading to the development of in vitro and in silico testing batteries which will in turn lead to a better evaluation of EDC exposure, moving the toxicological field forward and preventing adverse effects.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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