Perinatal exposure to endocrine disrupting chemicals and neurodevelopment: how articles of daily use influence the development of our children

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Abstract

Substances that interfere with the body's hormonal balance or their function are called endocrine disrupting chemicals (EDCs). Many EDCs are ubiquitous in the environment and are an unavoidable aspect of daily life, including during early embryogenesis. Developmental exposure to these chemicals is of critical relevance, as EDCs can permanently alter developmental programs, including those that pattern and wire the brain. Of emerging interest is how these chemicals may also affect the immune response, given the cross-talk between the endocrine and immune systems. As brain development is strongly dependent on hormones including thyroid, androgens, and estrogens, and can also be affected by immunomodulation, this complicated interplay may have long-lasting neurodevelopmental consequences. This review focuses on data available from human cohorts, *in vivo* models, and *in vitro* assays regarding the impact of EDCs after a gestational and/or lactational exposure, and how they may impact the immune system and/or neurodevelopment.

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Introduction

Development of the human central nervous system is a complex process that begins at approximately three weeks of gestation with the formation and closure of the neural tube, and concludes at approximately twenty years of age with the maturation of neural circuits [1]. During this near twenty-one-year period of development, an array of cellular processes occur [2] – progenitor cells differentiate into neurons that migrate radially, tangentially, and rostrally across the brain, often traveling amazing distances to their destination [3]. Glial cells myelinate axons, depositing a fatty sheath crucial to transduction of electrical signals; these signals stimulate the formation of neural networks, as cells across the brain forge new and complicated relationships [3]. These information processing networks are formed and modified, aided by the amazing plasticity of the developing brain. The study of neurodevelopment is quickly evolving in both the basic and applied sciences, and the use of new imaging, molecular, and functional technologies are illuminating discrete processes in the brain not yet understood [4]. In addition, novel and exciting systems biology studies aim to integrate so-called “big data” including anatomical, developmental and -omics studies, to further understand the association between molecular blueprints with function [4]. Because of this progress, these data also help reveal the intricacies of the developing central nervous system, as well as its potential vulnerabilities.

The devastating effects of thalidomide, an immunomodulator administered to pregnant women to combat morning sickness during the 1950s, unveiled that fetal development is a period uniquely susceptible to xenobiotics. Surviving children born to mothers taking this medication during the first trimester exhibited severe limb defects, craniofacial abnormalities, various organ malformations, and/or deafness [5]. This calamitous error in public health was considered an oversight in pharmaceutical regulation, as some European physicians recommended thalidomide for its off-label uses in their pregnant patients, assuming its safety [5]. The history of thalidomide holds several lessons for physicians and scientists alike. First,

we have learned in the last decades that exposure of both parents to environmental triggers before conception can permanently affect their children. Secondly, many xenobiotics and their metabolites can trespass the placenta and reach the fetus, and/or some may affect the fetus indirectly via chemical interactions at maternal organs including the placenta [6, 7]. Finally, a deep understanding of a chemical's toxicity, including its toxicokinetics, metabolism, and any consequent teratogenic effects, are crucial to ensure public safety. This not only applies to pharmaceuticals, but also to manufactured chemicals that are unintentionally released into the environment and/or are present in consumer products.

Endocrine disrupting chemicals (EDCs) are xenobiotics that interrupt hormone homeostasis and/or their cellular action in an organism [8]. As endocrine signaling directly controls pregnancy and embryonic development, understanding the health effects of EDCs is a public health concern. However, many manufactured chemicals have/will not undergo thorough *in vivo* toxicity testing, and many suspected EDCs are not necessarily under regulation to minimize human exposure [9]. It is undisputed that humans are exposed to these chemicals, and some of the most well-studied EDCs can be readily measured in amniotic fluid, cord blood, placental tissue, breast milk, and in the sera of fetuses, children, and adults. For example, bisphenol A (BPA) is a chemical widely used in the manufacturing of polycarbonate plastics and epoxy resins found in many consumer products, and is most notably weakly estrogenic *in vivo* [10]; BPA has been measured in human fetal serum at concentrations of 1-2 ng/ml [11]. For comparison purposes, endogenous serum estradiol in premenopausal women is approximately 0.03-0.90 ng/ml (30-800 pg/ml). [12]. So, while estradiol and BPA have differing estrogen receptor activation potencies [13, 14], the serum concentration of BPA is nonetheless concerning when compared to endogenous ligands like estradiol, which normally control cell signaling at picomolar concentrations [15]. BPA also accumulates in amniotic fluid and was reported to reach concentrations 5-fold higher than in maternal serum [11]. Therefore, the developing fetus or newborn can experience a greater exposure to EDCs, like BPA, than adults for several reasons. First, many environmental contaminants reach the fetus via

placental transfer [16, 17] and or after parturition via the breast milk [18]. Secondly, infants and toddlers explore their environment by crawling and have high hand-to-mouth behaviors, which increases their exposure to EDCs that may be found in house dust or toys [19, 20]. And finally, the expression and function of key molecules that regulate detoxification or elimination may be different during development as compared to adulthood, which can contribute to a greater overall body burden [21].

Within the last decade it has become clear that one of the most influential targets of environmental EDCs may be the brain, and that exposure perinatally and/or during childhood may be associated with neurodevelopmental disorders. As previously mentioned, the brain develops across many years; even when patterning of most brain structures may be near completion by birth, there are crucial developmental processes which occur after parturition. For example, the brain growth spurt occurs in humans from mid-gestation to approximately three years of age, with an estimated 5/6 of the spurt occuring after birth [22]. This attests to the enormous amount of cell proliferation, myelinogenesis, and other such pathways which are highly active during this period. It is important to acknowledge the pervasive believe in toxicology that the developing brain may be more susceptible to chemical insult due to an immature blood-brain barrier [23]. However, this is a contested argument, and elegant experiments in animal models and in human embryonic tissue have shown otherwise [24, 25]. Even though the blood-brain barrier is formed and active during early fetal development, the brain barriers are not impenetrable [24]. It is possible for environmental xenobiotics to cross both the blood and cerebrospinal fluid barriers by several mechanisms, including a case of “mistaken identity”, where some xenobiotics may resemble natural signaling molecules that are normally transported into the brain tissue. This is especially relevant considering some EDCs interact with nuclear receptors (agonists/antagonists), and thus have a chemical conformation like endogenous ligands that would normally be transported into the brain. Furthermore, EDCs may affect the brain by indirect mechanisms, and thus a chemical’s ability to cross the brain barriers is irrelevant. For example, maternal iodine deficiency during

pregnancy can lead to cretinism, a multisystem developmental disorder which includes intellectual disability due to maternal hypothyroidism [26]. While cretinism is now rare in developed countries, this example highlights how an environmental trigger (maternal diet) can indirectly, but severely, affect brain development and function [26]. Thus, it is possible for EDCs to first affect hormone concentrations in the periphery which then lead to reduced brain tissue hormone levels and consequently neurodevelopmental effects [27]. And finally, many EDCs likely affect multiple biological pathways simultaneously [28], and it is well understood that endocrine signals directly affect other processes important to brain development, including the immune system [29-31].

It is challenging to show that a single environmental exposure may affect brain development in children, as no one is exposed to a single chemical. We are all exposed to a complex cocktail of chemicals daily, and it often takes years for disease symptoms to manifest, which complicates the interpretation of how a single chemical may be responsible for a complex phenotype [32]. For example, neurodevelopmental disorders range from the more subtle learning disabilities, to the autism spectrum, to more severe effects like mental retardation and microcephaly. While the alarming rise of neurodevelopmental disorder diagnoses around the world is likely due in part to better detection and awareness, this alone does not likely account for the increase [33]. To help address the influence of the environment, new approaches seek to measure the entire “neuroexposome”, which includes factors like chemical exposure, dietary factors, and stress, to reveal how a cumulative insult may affect the developing brain [32]. As described by Heffernan and Hare 2018, three important scenarios must be considered when addressing how a chemical exposure may affect the brain: a) the direct effect of an exposure on early brain development which may directly cause disease, b) addressing the effects of a “multi-hit” or cumulative exposure an individual experiences throughout life, and c) acknowledging the concept of “sleeper-effects”, meaning that an early insult may result in the appearance of neurodevelopmental disorders later in life, including adulthood [32]. Addressing a concept outlined by Heffernan and Hare, our review will address a concept within

the umbrella of the “multi-hit hypothesis”. We will discuss perinatal exposure to EDCs that may also affect the immune system and explore how the interplay between endocrine and immune effects could be related to neurodevelopmental effects in both humans, animal models, and cell-based assays.

The Endocrine and Immune Systems both Influence Brain Development

It is well accepted that during adulthood, immune regulation in the brain helps to establish complex behaviors in mammals. For example, in mice T-helper cells ($CD4^+$ T) are recruited to the meninges and secrete interleukin-4 (IL-4) during learning tasks [34]. This localized IL-4 secretion induces the production of brain-derived neurotrophic factor by astrocytes, leading to improved spatial learning and memory [34]. While these data show how important immune cells are to normal brain function, there is growing evidence that the immune system also plays an important role during development. Work in laboratory animals and in humans have suggested that inappropriate inflammatory mechanisms, both systemically and in the brain itself, may influence the physiopathology of neurodevelopmental disorders through several proposed mechanisms. Among them are increased oxidative stress, reduced neurotrophic support, altered neurotransmitter metabolism, and blood-brain barrier disruption (reviewed in [35, 36]. So how would the endocrine system interact with these numerous and complex mechanisms related to the immune system? It is fascinating to consider that nearly all immune cells in the body express hormone receptors [29, 30]. This indicates that these cells can directly respond to hormonal cues, albeit by endogenous hormones or xenobiotics like EDCs [29, 30]. In support of this inference, it is well known that the immune system itself displays may sex-specific characteristics and changes across the lifespan in parallel to maturation or aging (i.e., puberty and menopause) [31]. Given this complex interplay between immune cells, hormones, and brain development, a newly emerging topic in the field of environmental toxicology is how the EDCs may interfere with both the endocrine and immune systems to impact brain development.

Evidence Correlating EDCs to Immune Dysfunction or Neurodevelopmental Disorders

An important step in human health risk assessment is to determine the potential hazard of a chemical, and then characterize the exposure of this compound in a population of interest. In this context epidemiological studies are crucial, as an exposure can be estimated from chemical concentration(s) in biofluids and/or tissues, and then compared to the incidence of disease. Here we discuss such evidence, where the internal dose of an EDC(s) is estimated in a population, and then compared to the incidence of immune alterations and/or neurodevelopmental disorders. However, it is important to recognize that most epidemiological data are correlative and do not show causation. There are also few published studies that have quantified an EDC exposure(s), immune system function, and neurodevelopmental disorders within the same cohort. To address the existing limitations in this epidemiological literature, we also provide evidence generated by *in vivo* and *in vitro* studies. These explorative and hypothesis-driven studies have provided intriguing data to suggest that EDCs may affect the immune system as well as the developing brain. For the sake of brevity, we will specifically focus on how three chemical classes (phthalates, perfluoroalkyl substances, and bisphenols) may be correlated to autism, attention deficit hyperactivity disorder, and learning disabilities, respectively. The data reviewed in the following section are summarized in further detail within Tables 1-3, and the hypotheses regarding how some EDCs may impact the brain via the immune system are summarized in Figure 1.

<u>Autism Spectrum Disorder (ASD)</u>

ASD and ASD-like behaviors are characterized by difficulties in verbal and nonverbal communication and are often accompanied by abnormalities in sensory processing. The risk of autism has been correlated to an increase in immune dysfunction, and this connection is of increasing interest. In women with preeclampsia, an especially severe disease where systemic inflammation is apparent, it has been shown that their children are estimated to be twice as likely to manifest with ASD [37]. Perhaps even more interestingly, it is established

that children that suffer from ASD are disproportionately affected by immune system dysfunction themselves, including an increased incidence of food and skin allergies [38]. Additional evidence also suggests that there may be an immune dysfunction/inflammation component in ASD, as markers of inflammation identified in the brain and cerebrospinal fluid of many ASD patients include Tumor Necrosis Factor (TNF), Interleukin-6 (IL-6) and Monocyte Chemotactic Protein-1 (MCP-1), the latter of which also is chemotactic for mast cells [39, 40]. So, while there is supporting evidence to suggest that ASD may have an etiology tied to the maternal and/or childhood immune system, is there any evidence to suggest that EDCs may also be correlated to this neurodevelopmental disorder?

Phthalates, or phthalate esters, are chemicals primarily used to soften plastic products and an estimated 11 billion pounds of phthalates are produced in manufacturing worldwide each year [41]. These chemicals are used in items like cosmetics, fragrances, food packaging, varnishes, and toys. Phthalates are readily metabolized in the body, and metabolites of this chemical class can be quantified in humans and livestock [41]. Phthalates are suspected endocrine disruptors, and an impressive body of literature has accumulated in animals and humans to suggest that male reproductive malformations, sperm damage, fertility impairment, female reproductive tract diseases, and precocious puberty are all correlated to phthalate exposure (see comprehensive toxicological profiles assembled for two phthalates by The Agency for Toxic Substances and Disease Registry (ASTDR) [42, 43]. In addition, immunomodulation is also an expected health effect. In a novel study recently reported, 1,074 mothers were recruited to investigate if phthalates exposure during late pregnancy may correlate with ASD and cognition in their children at 2-4 years of age. Given the potential interplay between phthalates, immune function, and neurodevelopment, this study also employed single nucleotide polymorphism (SNP) genotyping of their mothers. Specifically, 79 SNPs were assessed in the women that had been previously associated with oxidative stress pathways, and at 36 weeks of pregnancy urinary phthalate concentrations were quantified [44]. Their children were consequently evaluated for ASD, cognition, and ADHD by various

metrics including the Bayley Scales of Infant and Toddler Development 3rd edition (BAYLEY-III) and professional diagnosis by at least two professionals. Results of this study showed strong associations between higher maternal phthalate exposure and an increased risk of ASD or ASD traits [44]. Additionally, children born to mothers positive for certain SNP variants were also associated with an increased risk of each neurodevelopmental effect investigated regardless of phthalate exposure [44]. Interestingly, those infants born to mothers with both a higher-risk gene score and a high phthalate exposure were at substantially elevated risk of neurodevelopmental outcomes, especially ASD [44]. While the candidate SNPs investigated in this study are not necessarily causal to increased oxidative stress, it nonetheless suggests that a genetic susceptibility to an abnormal immune response combined with phthalates exposure(s) during late pregnancy may increase the possibility of the child with ASD-like behaviors.

While there is only one human study that investigates both immune variables and ASD, there are several that investigate ASD alone. In Patti et al*.* 2021, the authors examined how urinary concentrations of phthalates during pregnancy may correlate to ASD in two different human cohorts. The authors showed that in a cohort of 276 mother-child pairs, increasing gestational urinary concentrations of monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), monoisobutyl phthalate (MiBP), mono-(3-carboxypropyl) phthalate (MCPP), and summed di-(2-ethylhexyl) phthalate metabolites (∑DEHP) were positively associated with increasing percentiles of Social Responsiveness Scale t-scores in children aged 4-8 years [45]. This suggests increased ASD behaviors in these children. However, it is important to note that in the second human cohort, the authors discovered an inverse or null association between maternal and childhood SRS t-scores, with the exception of the metabolite MBzP which also showed a positive association [45]. Reporting similar findings, a longitudinal study recently published also measured urinary phthalate concentrations in pregnant women during early and late pregnancy, and then evaluated children's behavior by both SRS and Behavioral Assessment System for Children (BASC-2), another test that measures several dimensions

of behavior [42]. This study employed 500 mothers and their children, and examined potential mixture effects, in order to infer any potential interactions or cumulative consequences of the total estimated phthalate exposure [46]. Their results also show increased SRS scores in both male and female children, which were positively associated to phthalate exposure during gestation [46]. In addition, they showed that boys displayed increased externalizing behavior, an established trait of autism, as a function of late gestational phthalate exposure [47]. This study also showed the strongest association between MBzP and MCPP and neurodevelopmental abnormalities, which is complimentary to other epidemiological investigations [45, 46, 48]. So, while increasing evidence suggests that phthalates may be associated with ASD, this is not necessarily a causative relationship. There are other studies that show a positive association and/or correlation, but others that show an inverse or null relationship [49].

In accordance to observation in human cohorts, numerous *in vitro* and *in vivo* studies suggest negative consequences of phthalates on neurodevelopment and behavior. Two very recent publications focused on how DEHP-exposure in male mice of two different strains may affect autism-like behavior, as well as inflammatory responses in the brain and peripheral cells [50, 51]. They found that early life exposure (three weeks of age) to DEHP leads to impaired social interaction and increased repetitive behavior – signs for an autism-like behavior – in BTBR, and to a much lesser extent in C57BL/6 mice. No signs of autism-like behavior were observed in C57BL/6 mice exposed later in life (six weeks of age) even at a higher DEHP-concentration [50]. This demonstrates that the window of exposure and genetic background are crucial for the outcome of a study and the interpretation of the results. Nadeem and colleagues investigated biomarkers of inflammatory responses in the brain and in the periphery in two different publications [46, 47]. Interestingly, they found evidence of both oxidative stress and epigenetic alterations in both compartments in the BTBR mice after DEHP exposure [50, 51]. Despite DEHP inducing oxidative stress in both mouse strains, C57BL/6 mice showed a stronger antioxidative response in comparison to BTBR mice [50]. In accordance, the

elevation of inflammatory immune markers, such as the expression of IL-6, TNF-α, and MCP-1 in cortex and peripheral dendritic cells was much more pronounced in BTBR mice than in C57BL/6 mice. This work demonstrating strain differences and thus genetic susceptibility is reminiscent of the epidemiological study described above, where children born to women with certain SNP markers in addition to increased phthalate exposure were at increased risk of ASD.

In addition to these studies that focus directly on how phthalate exposure may impact both the immune system and development of ASD-like traits in animal models, there are other publications which investigate other aspects of neurodevelopment. Some of the reported effects are quite severe, including a reduction in both brain size and neuron and synapse numbers in rat and zebrafish [52, 53]. While the severity of the neurodevelopmental effects are likely dose-dependent observations, there are also instances of more nuanced and translational neurodevelopmental outcomes in addition to ASD. For example, a perinatal exposure to DEHP in mice was shown to reduce locomotor behavior, increase anxiety, and impair spatial and short-term recognition in mice [54, 55]. Like observations reported in some epidemiological studies, these effects were sometimes sex-dependent. In a study by Kougias and colleagues, male rats exposed perinatally to a mixture of phthalates were found to have abnormal social behavior, whereas female rats were unaffected [56]. The potential mechanisms of phthalate-induced neurotoxicity has been hypothesized to include the induction of oxidative stress, mitochondrial dysfunction, apoptosis, reduced neuronal proliferation, and DNA damage in neurons [52, 57, 58] (see Figure 1 for example cellular mechanisms). The latter was especially shown to be associated with impaired neurogenesis as well as spatial learning and memory disabilities of mice that were exposed *in utero* to DBP [59]. Phthalates may also influence neurodevelopment by targeting immune cell function, which can then lead to brain damage. In support of this hypothesis, phthalates have been shown to induce both systemic and neuroinflammation *in vitro* and *in vivo*. For instance, macrophages secrete significantly higher levels of TNF-α, IL-6 or IL-1β upon stimulation with

DEHP [60]. While the precise mechanism of this observation is not yet fully understood, a recent study revealed that phthalates may lead to oxidative stress and interfere with epigenetic processes, namely Sirtuin activity, which results in NF-κB-hyperacetylation and the induction of a pro-inflammatory reaction [61]. A prolonged and/or higher concentration of phthalate exposure may induce oxidative damage and thus result in suboptimal immune cell function, rather than an inflammatory response, as shown in a zebrafish model [62]. As both placental and neuro- inflammation is linked to autism onset [37, 39], these are intriguing observations. Even though the cellular and molecular mechanisms behind phthalate action *in vivo* are not fully understood, it is reasonable to consider that this class of EDCs may also interact with the immune system and potentially the brain, and these hypotheses must be addressed by ongoing research. The data reviewed in this section are summarized in Table 1.

Attention Deficit and Hyperactivity Disorder (ADHD)

Symptoms of ADHD come in many forms but is generally characterized by persistent inattention and/or hyperactivity that interferes with daily life. ADHD is relatively difficult to diagnose in very young children, and the median age at diagnosis is approximately six years [63]. There is a good body of evidence that suggests inflammation during development is an underlying pathophysiology that is correlated to ADHD (see review by Leffa et al. 2018 [36]); there is also growing evidence that developmental EDC exposure(s) during pregnancy and childhood may increase the risk of developing this ADHD. Unfortunately, there are few observational studies that examine immune markers, ADHD, and an EDC exposure simultaneously. However, there is growing interest in both the immune and neurodevelopmental effects of a chemical class called per- and polyfluoroalkyl substances (PFAS). PFAS are both hydro- and lipophobic chemicals manufactured as surfactants, and are utilized to create nonstick cookware, food packaging, rainproof clothing, and stain repellant products including textiles [64]. PFAS also contaminate drinking water and soil around the world, and many chemicals of this class are categorized as persistent organic pollutants [64].

These chemicals are highly stable in both the environment and within the body, and some PFAS have an estimated human half-life greater than five years [65]. Many well-designed and statistically powerful epidemiologically studies have been executed to study potential human health effects of PFAS, and endocrine disruption, serum dyslipidemia, hepatotoxicity, and immune abnormalities are some of the most well recognized endpoints in the literature [66].

Several PFAS have been correlated to an increased incidence of autoimmune diseases in adults, with a probable link established between perfluorooctanoic acid (PFOA, also referred to as C8) and ulcerative colitis [67, 68]. There is also growing evidence that various PFAS are associated with the suspected inflammatory condition preeclampsia [69-71], as well as immunomodulation in children [72-75] (also see Table 2 for additional details). More recent work has specifically investigated ADHD in children following a developmental exposure to PFAS. Lenters et al. 2019 measured 27 different persistent organic pollutants in breast milk and later correlated the estimated exposure(s) to the incidence of ADHD in their children (72). Of the two PFAS investigated, breastmilk concentrations of perfluoroctane sulfonic acid (PFOS) was shown to increase the risk of developing ADHD (71). This association was stronger for girls than boys [76]. Another study also showed that increasing maternal serum concentrations of PFOS and perfluorononanoic acid (PFNA) correlate to 50-80% more neurobehavioral symptoms related to hyperactive-impulsive type ADHD, and prenatal PFNA was associated with increased risk of ADHD [77]. In a different publication where several PFAS were measured in umbilical cord blood, PFNA was associated with ADHD-like symptoms in 7-year old children [78]. While these data suggest that perinatal exposure to PFAS may be associated with ADHD, other studies have shown null results [79-82]. Nonetheless, each study design was different with its own strengths and weaknesses, and additional epidemiological work is needed.

The mechanism of PFAS-induced neurotoxicity is not yet fully understood. *In vitro* studies revealed that these substances can induce oxidative stress, apoptosis [83, 84] and a Ca^{2+}-

dependent pro-inflammatory response in microglia [85]. More recently, Tukker and colleagues demonstrated that PFOS and PFOA might impair the inhibitory functions of the CNS and lead to hyperexcitation by an antagonistic, non-competitive effect on the human $\alpha_1\beta_2\gamma_2$L $GABA_A$ receptor [86]. The disturbance of the GABAergic system is associated with a number of neurodevelopmental diseases, and suggests that this mechanism may underlie some observations in rodent and epidemiological studies [87]. However, although Tukker et al. 2020 show that both PFAS inhibited the GABA-evoked recurrent of the receptor, only high concentrations of PFOS led to an increase in the network activity of rat primary cortical cultures (82). Moreover, there was an unexpected decrease of the network activity in human neuron cultures after treatment with PFOS, which is contrary to results using rat cortical cultures. The authors argue that either compensatory mechanisms at the cellular level or additional effects of the investigated chemicals that overlay the inhibitory effect on the $\alpha_1\beta_2\gamma_2$L $GABA_A$ receptor might be responsible for this phenomenon [86]. However, it also important to consider that these studies utilize high concentrations of PFAS in culture, and it is unclear how this translates to a biologically relevant exposure. In accordance with human trials, *in vivo* studies in zebrafish revealed the hyperactivity-inducing potential of some PFAS such as PFOS and perfluorohexanoic acid (PFHxA) [88-90]. Moreover, PFAS were able to modulate molecular markers such as the expression of genes related to neurodevelopment in this model system [91]. A study by Viberg et al*.* 2013 has also shown that even a single dose of the PFAS perfluorohexane sulphonic acid (PFHxS) during a critical window of neurodevelopment in newborn mice has consequences on the behavior later in adulthood, including altered function of the cholinergic system [92]. But like all other living organisms, humans are exposed to a complex mixture of chemicals in their environment. Thus, particularly in the last years, scientific questions have addressed PFAS mixture effects, including potential interactions between different chemicals *in vivo*. A study in zebrafish larvae showed that exposure to different PFAS may have opposing effects on behavior: PFOA exposure lead to an increase in swimming during dark periods in exposed fish, which is considered an anxiety-like phenotype (89). On the contrary, PFHxS decreased swimming in this light-dark assay,

suggesting an anti-anxiety like effect in developmentally exposed fish [93]. Moreover, a co-exposure to a mixture of PFAS induced behavioral effects that were less severe as compared to a single PFAS treatment [93]. In a complimentary zebrafish study from a different laboratory, co-exposure of larvae to six different PFAS induced anxiety-like behaviors, but only PFOS was deemed responsible for the effect of the complex mixture [91]. Importantly, not only chemicals within the same class are able to interact with one another *in vivo*. Reardon et al. 2019 showed that either PFOS or methylmercury (MeHg) induced hyperactivity in developmentally exposed rats independently, and both chemicals impacted concentrations of metabolites such as GABA, taurine, and other amino acids vital for normal brain function. However, rats co-exposed to a mixture of both substances (PFOS and MeHg) did not lead to any observed behavioral or molecular effects [94]. Collectively, these studies suggest that PFAS may have neurotoxic potential, and may affect neurodevelopment to at least some extent. However, effects of different substances – even from the same chemical class – might elicit different and even unexpected outcomes. Furthermore, neurodevelopmental outcomes may not be predictable by assuming the addition of individual chemical effects. Instead, some phenotypes may be additive following exposure to a complex mixture, while others may result in a diminished overall effect. Given the long half-life of many PFAS and their ubiquitous use in household items and in food/water sources, further research is greatly needed to show how these chemicals may affect the developing brain. The data reviewed in this section are summarized in Table 2.

Learning disabilities:

Learning disabilities are a complex and heterogenous set of disorders that manifest as difficulties in acquiring knowledge and skills to the level expected of those of the same age, especially when not associated with a physical handicap [95]. For example, a child may struggle in the classroom with a specific subject or schooling in general, without presenting with severe intellectual impairment [95]. Learning disabilities are measured in epidemiological studies by various methods including intelligent quotient (IQ) scores, although IQ has been

criticized as an inappropriate measure as many affected children with learning disabilities do not suffer from intellectual dysfunction [96]. Therefore, some studies also utilize metrics like the Learning Disability Evaluation Scale (LDES), as it encompasses specific measures related to thinking, speaking, reading, writing, and mathematical calculations [97]. The etiology of learning disabilities is likely due to several contributing factors, and while they are not uncommon nor debilitating, they nonetheless are associated with an economic and emotional burden for affected families.

Immune dysregulation during pregnancy and childhood development are associated with an increased incidence of learning disabilities in some populations. In an impressive longitudinal study, Rudolph et al. show that variations in maternal IL-6 concentrations during pregnancy are correlated with abnormal memory in young children. Specifically, increased maternal IL-6 during the third trimester of pregnancy showed the strongest association, and the authors suggest that increased systemic immune activation during this period is associated with decreased working memory performance of offspring at 2 years of age [98]. The authors also demonstrate abnormal neuroconnectivity in these children [98]. With regards to EDCs that may affect the immune system and are also correlated to learning disabilities, BPA is a notable example. BPA, as previously mentioned, is a plasticizer with a large portion of human exposure attributed to consumption of contaminated foods and beverages [99]. BPA interferes with a variety of molecular and physiological pathways, including but not limited to steroid and thyroid signaling, and has been shown to induce DNA damage in mice even at very low doses (reviewed in Rochester, 2013 [100]). Interestingly, several epidemiological studies have provided evidence that BPA may be pro-inflammatory during pregnancy, as well as associated with learning disabilities in children. In a manuscript by Ferguson et al. 2019, urine BPA concentrations were measured, along with both urine and plasma concentration of pro-oxidative and pro-inflammatory biomarkers at 4 intervals during pregnancy (median 10, 18, 26, and 35 weeks gestation, respectively). This study found that increased concentrations of BPA in urine were positively associated with an increase in two biomarkers of oxidative stress

(8-hydroxydeoxyguanosine and 8-isoprostane). Additionally, plasma concentrations of the pro-inflammatory cytokine IL-6 were also increased across pregnancy [101]. While the authors did not measure any neurodevelopmental outcomes in the children, other work has correlated BPA exposure with learning disabilities. One study showed that children between 8-11 years of age that were exposed to either low or high concentrations of BPA were more likely to have issues with listening, as determined by the Learning Disability Evaluation Scale (LDES) [102]. The quadratic association, as opposed to a linear relationship between dose and effect, suggests a non-monotonic dose response, a characteristic of BPA exposure in some animal toxicity studies [103, 104]. A nonmonotonic dose response is of especial relevance, as it suggests the potential for low dose effects, some of which may occur at environmentally relevant exposures like those suggested in the Hong et al. 2013 study.

Bisphenol A and learning disabilities – focus on influence of the microbiota

BPA likely acts via various mechanisms to modulate immune and central nervous system physiology. For example, BPA treatment of microglia cells in culture leads to cell activation and production of cytokines (TNF-α, IL-6), an effect that was at least partially mediated by estrogen receptor signaling [105]. This is in accordance with the correlation between BPA concentrations in the urine and IL-6 plasma levels found in humans, suggesting that bisphenol exposure may lead to immune dysregulation and/or inflammation. Several other studies have also focused on the immunological consequences of BPA-exposure and revealed effects on both the innate and adaptive immune responses. For instance, BPA exposure *in vitro* leads to a shift of the M1/M2-macrophage balance towards M1 [106], and of the Treg/Th17-balance towards Th17 *in vivo* [107], further emphasizing its pro-inflammatory potential. Recently, Kaur et al. 2021 found differences in hypothalamic micro (miR)/small RNAs that were related to response to nerve growth factor, regulation of innate immune response and positive regulation of defense response upon perinatal BPA treatment [108]. This might be a potential mechanism that connects BPA actions on several different cells in a specific environment. This observed effect was sex-dependent and the group also correlated this exposure with behavioral

parameters in developmentally exposed mice [108]. Collectively, BPA acts on different cell types and influences their physiology via several pathways. The interaction of these pathways and the relation between immune and nervous system especially with respect to the development of behavior emerges as a very relevant question for further investigations.

A new scientific field that is of high relevance for neurodevelopment has recently emerged. The so-called ‘microbiota-gut-brain-axis’ describes the bidirectional communication between the intestinal microbiome and the central nervous system, and encompasses neuronal, chemical, and immunological signals linking the microbiota to functional consequences (for review see Morais et al. 2021, [109]). Several studies specifically disclosed the microbiota as a potential target for EDCs. BPA exposure was shown to induce dysbiotic microbial alterations, such as a loss of overall diversity and alterations of specific taxonomical units in zebrafish as well as rodents, which were accompanied by increased intestinal permeability and altered immune responses [110-112]. A few studies also focused on consequences of EDC-induced microbiota alterations on neurodevelopment. For instance, Kaur and colleagues found correlations between microbiota and stereotypical and repetitive behaviors in California mice after exposure to BPA and genistein during gestation and lactation [113]. However, other behaviors reminiscent of human neurodevelopmental disorders such as impaired spatial learning or memory were not affected upon exposure to the same chemical mixture [113]. In a zebrafish model, BPA was found to induce dysbiosis and influence on intestinal levels of serotonin, an important neurotransmitter, in a sex-dependent manner [111]. A similar study performed in zebrafish also shows influence of early developmental exposure to several bisphenols on microbiota, but no effect was observed on behavior [114]. As research on the impact of EDCs on the microbiota with respect to neurodevelopmental consequences has only begun, it is clearly a new and exciting area of investigation. However, several important variables such as the vulnerable window(s) of exposure, species differences, and the influence of sex need to be addressed in the future. Moreover, defining the microbiome and its dysbiosis is not clear-cut in either animals or humans, especially as the microbiome is

readily influenced by the environment including diet [115]. These dynamics make study design and thus data interpretation complicated.

Many of the effects mentioned above were sex-dependent. Therefore, some studies particularly utilized models that display strongly sex biased behaviors, such as Deer mice. These mice have a well-defined steroid-dependent behavior and maintain naturally occurring sex differences despite housing in standardized laboratory environments. Two studies of Jašarević et al. 2011 and 2013 described that perinatal exposure to BPA leads to dose-dependent impairments in spatial learning, increased anxiety and reduced exploratory behaviors in males but not females [116, 117]. Another model organism, the California mouse, is monogamic and both parents invest in offspring care. BPA-treatment led to decreased care by the females whereas males were less affected [118]. In the same model, Williams found no effect on spatial navigational skills in either males or females, however BPA exposure abolished the natural sex differences in exploratory behavior [119]. Altogether, these studies demonstrate that BPA can exert its neurotoxic effects in a sex-dependent manner, which must be considered when designing and performing *in vivo* studies, and when analyzing epidemiological data. In all, a body of data exists that suggests bisphenols have multiple mechanisms of action *in vivo* and are correlated to immune dysfunction and/or neurobehavioral effects in some studies (see Table 3).

Summary

While the precise health effects of environmental EDCs are not always clear, there is evidence that exposure to some compounds may lead to abnormal brain development and/or function. Given the known crosstalk between the endocrine and immune systems, there is also a growing interest in understanding how immune responses may also contribute to observed neurodevelopmental effects. The regulation and use of chemicals around the world are a complicated issue, and details of this process are out of scope of this review (see Kassotis et al. 2020 for further information). But in short, many compounds used in commercial

manufacturing, household items, and even cosmetics are not necessarily required to undergo *in vivo* developmental and/or neurotoxicity testing before they are marketed. Therefore, minimizing unwanted risk is often the responsibility of the consumer. The following recommendations are examples to reduce personal exposure to EDCs, and these have also been outlined by The Endocrine Society to educate physicians and their patients [120]. To minimize exposure to EDCs in drinking water, carbon filtration is a simple and cost-effective method to removes many contaminants including PFAS. Given that plastics and coated aluminum cans are known to leech EDCs like bisphenols and phthalates, replacing plastic products with glass or a similar substance is a simple yet effective switch. This includes replacing plastic food storage containers, plastic baby bottles, plastic toys, and avoiding bottled beverages and canned food. When eating fresh fruits and vegetables, the preferred choice for both nutrition and to avoid EDCs, thoroughly washing and/or soaking produce is a simple way to remove any residual pesticides. This should be performed for both organic and conventionally grown produce, as organic produce is often still sprayed with herbicides and pesticides [121]. While much is unknown regarding EDCs, physicians and patients can act to offset adverse neurodevelopmental effects, regardless of the cause. We greatly emphasize that the brain is the most amazingly plastic organ in the body, and as its development occurs across a protracted period, there is ample opportunity to form and modify neural circuity with stimulation and enrichment. Thus, early identification and professional intervention are key to offset the effects of many learning and behavioral disorders, and physicians can encourage families to optimize their own child's neurodevelopment from a very early age. This may be as simple as reading together, exploring nature, and/or listening to music. While this may seem like banal advice, it has been shown that increased family interaction greatly benefits children diagnosed with neurodevelopmental disorders [122]. In conclusion, there are many data gaps in epidemiological and experimental studies regarding the neurodevelopmental effects of EDCs. Whether there is also a link to immunomodulation as well remains to be fully explored, but there are many important hypotheses that should be addressed in the future.

Practice Points

- Many suspected or known EDCs are measurable in human biofluids and tissues.
- As the endocrine and immune systems interact, EDCs could potentially impact the central nervous system of children by various mechanisms.
- It is possible to reduce exposure to EDCs through lifestyle adjustments.

Research Agenda

- Further epidemiological and experimental research is needed to fully understand how EDCs could interact with the immune system to affect brain development.
- This includes experiments to model human exposures (e.g. mixtures of chemicals, internal doses comparable to humans, etc).

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Declaration of competing interests

The authors have no competing interests to declare. All views presented in this manuscript are the authors' opinions and do not reflect policy or formal recommendations. This document has been subjected to review by the United States Environmental Protection Agency's Center for Public Health and Environmental Assessment and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

[1] Detrait ER, George TM, Etchevers HC, Gilbert JR, Vekemans M, Speer MC. Human neural tube defects: developmental biology, epidemiology, and genetics. Neurotoxicol Teratol. 2005;27:515-24.

[2] Johnson MH. Functional brain development in humans. Nat Rev Neurosci. 2001;2:475-83.

[3] Stiles J, Jernigan TL. The basics of brain development. Neuropsychol Rev. 2010;20:327-48.

[4] Briscoe J, Marin O. Looking at neurodevelopment through a big data lens. Science. 2020;369.

[5] Vargesson N. Thalidomide-induced teratogenesis: history and mechanisms. Birth Defects Res C Embryo Today. 2015;105:140-56.

[6] Ward RM, Varner MW. Principles of Pharmacokinetics in the Pregnant Woman and Fetus. Clin Perinatol. 2019;46:383-98.

[7] Blanco-Castaneda R, Galaviz-Hernandez C, Souto PCS, Lima VV, Giachini FR, Escudero C, et al. The role of xenobiotic-metabolizing enzymes in the placenta: a growing research field. Expert Rev Clin Pharmacol. 2020;13:247-63.

[8] Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 2015;36:E1-E150.

[9] Kassotis CD, Vandenberg LN, Demeneix BA, Porta M, Slama R, Trasande L. Endocrine-disrupting chemicals: economic, regulatory, and policy implications. Lancet Diabetes Endocrinol. 2020;8:719-30.

[10] Vom Saal FS, Vandenberg LN. Update on the Health Effects of Bisphenol A: Overwhelming Evidence of Harm. Endocrinology. 2021;162.

[11] Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum Reprod. 2002;17:2839-41.

[12] Stanczyk FZ, Clarke NJ. Measurement of estradiol--challenges ahead. J Clin Endocrinol Metab. 2014;99:56-8.

[13] Kim HS, Han SY, Yoo SD, Lee BM, Park KL. Potential estrogenic effects of bisphenol-A estimated by in vitro and in vivo combination assays. J Toxicol Sci. 2001;26:111-8.

[14] Matthews JB, Twomey K, Zacharewski TR. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. Chem Res Toxicol. 2001;14:149-57.

[15] Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr., Lee DH, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev. 2012;33:378-455.

[16] Konkel L. Lasting Impact of an Ephemeral Organ: The Role of the Placenta in Fetal Programming. Environ Health Perspect. 2016;124:A124-9.

[17] Vizcaino E, Grimalt JO, Fernandez-Somoano A, Tardon A. Transport of persistent organic pollutants across the human placenta. Environ Int. 2014;65:107-15.

[18] Mead MN. Contaminants in human milk: weighing the risks against the benefits of breastfeeding. Environ Health Perspect. 2008;116:A427-34.

[19] Reed KJ, Jimenez M, Freeman NC, Lioy PJ. Quantification of children's hand and mouthing activities through a videotaping methodology. J Expo Anal Environ Epidemiol. 1999;9:513-20.

[20] Development of EC-oa. Estimating Mouthing Exposure in Children - Compilation of Case Studies. In: Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party on Chemicals P, and Biotechnology editor.2019. p. 1-75.

[21] Ginsberg G, Slikker W, Jr., Bruckner J, Sonawane B. Incorporating children's toxicokinetics into a risk framework. Environ Health Perspect. 2004;112:272-83.

[22] Dobbing J, Sands J. Quantitative growth and development of human brain. Arch Dis Child. 1973;48:757-67.

[23] Ek CJ, Dziegielewska KM, Habgood MD, Saunders NR. Barriers in the developing brain and Neurotoxicology. Neurotoxicology. 2012;33:586-604.

[24] Saunders NR, Dziegielewska KM, Mollgard K, Habgood MD. Recent Developments in Understanding Barrier Mechanisms in the Developing Brain: Drugs and Drug Transporters in Pregnancy, Susceptibility or Protection in the Fetal Brain? Annu Rev Pharmacol Toxicol. 2019;59:487-505.

[25] Johansson PA, Dziegielewska KM, Liddelow SA, Saunders NR. The blood-CSF barrier explained: when development is not immaturity. Bioessays. 2008;30:237-48.

[26] Chen ZP, Hetzel BS. Cretinism revisited. Best Pract Res Clin Endocrinol Metab. 2010;24:39-50.

[27] O'Shaughnessy KL, Gilbert ME. Thyroid disrupting chemicals and developmental neurotoxicity - New tools and approaches to evaluate hormone action. Mol Cell Endocrinol. 2020;518:110663.

[28] La Merrill MA, Vandenberg LN, Smith MT, Goodson W, Browne P, Patisaul HB, et al. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. Nat Rev Endocrinol. 2020;16:45-57.

[29] Bereshchenko O, Bruscoli S, Riccardi C. Glucocorticoids, Sex Hormones, and Immunity. Front Immunol. 2018;9:1332.

[30] Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. Hum Reprod Update. 2005;11:411-23.

* [31] Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16:626-38.

* [32] Heffernan AL, Hare DJ. Tracing Environmental Exposure from Neurodevelopment to Neurodegeneration. Trends Neurosci. 2018;41:496-501.

[33] Hansen SN, Schendel DE, Parner ET. Explaining the increase in the prevalence of autism spectrum disorders: the proportion attributable to changes in reporting practices. JAMA Pediatr. 2015;169:56-62.

[34] Derecki NC, Cardani AN, Yang CH, Quinnies KM, Crihfield A, Lynch KR, et al. Regulation of learning and memory by meningeal immunity: a key role for IL-4. J Exp Med. 2010;207:1067-80.

* [35] Jiang NM, Cowan M, Moonah SN, Petri WA, Jr. The Impact of Systemic Inflammation on Neurodevelopment. Trends Mol Med. 2018;24:794-804.

[36] Leffa DT, Torres ILS, Rohde LA. A Review on the Role of Inflammation in Attention-Deficit/Hyperactivity Disorder. Neuroimmunomodulation. 2018;25:328-33.

[37] Walker CK, Krakowiak P, Baker A, Hansen RL, Ozonoff S, Hertz-Picciotto I. Preeclampsia, placental insufficiency, and autism spectrum disorder or developmental delay. JAMA Pediatr. 2015;169:154-62.

* [38] Xu G, Snetselaar LG, Jing J, Liu B, Strathearn L, Bao W. Association of Food Allergy and Other Allergic Conditions With Autism Spectrum Disorder in Children. JAMA Netw Open. 2018;1:e180279.

[39] Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun. 2012;26:383-92.

[40] Theoharides TC, Angelidou A, Alysandratos KD, Zhang B, Asadi S, Francis K, et al. Mast cell activation and autism. Biochim Biophys Acta. 2012;1822:34-41.

[41] Production LCfS. Phthalates and Their Alternatives: Health and Environmental Concerns. Technical Briefing. 2011.

[42] The Agency for Toxic Substances and Disease Registry (ATSDR) USDoHaHS. Toxicological Profile for Di(2-Ethylhexyl)Phthalate (DEHP). 2019, Draft for Public Comment.

[43] The Agency for Toxic Substances and Disease Registry (ATSDR) USDoHaHS. Toxicological Profile For DI-n-Butyl Phthalate 2001.

* [44] Ponsonby AL, Symeonides C, Saffery R, Mueller JF, O'Hely M, Sly PD, et al. Prenatal phthalate exposure, oxidative stress-related genetic vulnerability and early life neurodevelopment: A birth cohort study. Neurotoxicology. 2020;80:20-8.

[45] Patti MA, Newschaffer C, Eliot M, Hamra GB, Chen A, Croen LA, et al. Gestational Exposure to Phthalates and Social Responsiveness Scores in Children Using Quantile Regression: The EARLI and HOME Studies. Int J Environ Res Public Health. 2021;18.

[46] Day DB, Collett BR, Barrett ES, Bush NR, Swan SH, Nguyen RHN, et al. Phthalate mixtures in pregnancy, autistic traits, and adverse childhood behavioral outcomes. Environ Int. 2021;147:106330.

[47] Shea N, Payne E, Russo N. Brief Report: Social Functioning Predicts Externalizing Problem Behaviors in Autism Spectrum Disorder. J Autism Dev Disord. 2018;48:2237-42.

[48] Oulhote Y, Lanphear B, Braun JM, Webster GM, Arbuckle TE, Etzel T, et al. Gestational Exposures to Phthalates and Folic Acid, and Autistic Traits in Canadian Children. Environ Health Perspect. 2020;128:27004.

[49] Shin HM, Schmidt RJ, Tancredi D, Barkoski J, Ozonoff S, Bennett DH, et al. Prenatal exposure to phthalates and autism spectrum disorder in the MARBLES study. Environ Health. 2018;17:85.

* [50] Nadeem A, Ahmad SF, Al-Harbi NO, Attia SM, Bakheet SA, Alsanea S, et al. Aggravation of autism-like behavior in BTBR T+tf/J mice by environmental pollutant, di-(2-ethylhexyl) phthalate: Role of nuclear factor erythroid 2-related factor 2 and oxidative enzymes in innate immune cells and cerebellum. Int Immunopharmacol. 2021;91:107323.

[51] Nadeem A, Al-Harbi NO, Ahmad SF, Alhazzani K, Attia SM, Alsanea S, et al. Exposure to the plasticizer, Di-(2-ethylhexyl) phthalate during juvenile period exacerbates autism-like behavior in adult BTBR T + tf/J mice due to DNA hypomethylation and enhanced inflammation in brain and systemic immune cells. Prog Neuropsychopharmacol Biol Psychiatry. 2021;109:110249.

[52] Xu S, Zhang H, Pao PC, Lee A, Wang J, Suen Chan Y, et al. Exposure to phthalates impaired neurodevelopment through estrogenic effects and induced DNA damage in neurons. Aquat Toxicol. 2020;222:105469.

[53] Kougias DG, Sellinger EP, Willing J, Juraska JM. Perinatal Exposure to an Environmentally Relevant Mixture of Phthalates Results in a Lower Number of Neurons and Synapses in the Medial Prefrontal Cortex and Decreased Cognitive Flexibility in Adult Male and Female Rats. J Neurosci. 2018;38:6864-72.

[54] Xu X, Yang Y, Wang R, Wang Y, Ruan Q, Lu Y. Perinatal exposure to di-(2-ethylhexyl) phthalate affects anxiety- and depression-like behaviors in mice. Chemosphere. 2015;124:22-31.

[55] Barakat R, Lin PC, Park CJ, Best-Popescu C, Bakry HH, Abosalem ME, et al. Prenatal Exposure to DEHP Induces Neuronal Degeneration and Neurobehavioral Abnormalities in Adult Male Mice. Toxicological sciences : an official journal of the Society of Toxicology. 2018;164:439-52.

[56] Kougias DG, Cortes LR, Moody L, Rhoads S, Pan YX, Juraska JM. Effects of Perinatal Exposure to Phthalates and a High-Fat Diet on Maternal Behavior and Pup Development and Social Play. Endocrinology. 2018;159:1088-105.

[57] Wojtowicz AK, Szychowski KA, Wnuk A, Kajta M. Dibutyl Phthalate (DBP)-Induced Apoptosis and Neurotoxicity are Mediated via the Aryl Hydrocarbon Receptor (AhR) but not by Estrogen Receptor Alpha (ERalpha), Estrogen Receptor Beta (ERbeta), or Peroxisome Proliferator-Activated Receptor Gamma (PPARgamma) in Mouse Cortical Neurons. Neurotox Res. 2017;31:77-89.

[58] Lee W, Cho JH, Lee Y, Lee S, Kim DH, Ha S, et al. Dibutyl phthalate impairs neural progenitor cell proliferation and hippocampal neurogenesis. Food Chem Toxicol. 2019;129:239-48.

[59] Levie D, Korevaar TIM, Bath SC, Dalmau-Bueno A, Murcia M, Espada M, et al. Thyroid Function in Early Pregnancy, Child IQ, and Autistic Traits: A Meta-Analysis of Individual Participant Data. J Clin Endocrinol Metab. 2018;103:2967-79.

[60] Nishioka J, Iwahara C, Kawasaki M, Yoshizaki F, Nakayama H, Takamori K, et al. Di-(2-ethylhexyl) phthalate induces production of inflammatory molecules in human macrophages. Inflamm Res. 2012;61:69-78.

[61] Park MH, Gutierrez-Garcia AK, Choudhury M. Mono-(2-ethylhexyl) Phthalate Aggravates Inflammatory Response via Sirtuin Regulation and Inflammasome Activation in RAW 264.7 Cells. Chem Res Toxicol. 2019;32:935-42.

[62] Xu H, Dong X, Zhang Z, Yang M, Wu X, Liu H, et al. Assessment of immunotoxicity of dibutyl phthalate using live zebrafish embryos. Fish Shellfish Immunol. 2015;45:286-92.

[63] Visser SN, Danielson ML, Bitsko RH, Holbrook JR, Kogan MD, Ghandour RM, et al. Trends in the parent-report of health care provider-diagnosed and medicated attention-deficit/hyperactivity disorder: United States, 2003-2011. J Am Acad Child Adolesc Psychiatry. 2014;53:34-46 e2.

[64] Brase RA, Mullin EJ, Spink DC. Legacy and Emerging Per- and Polyfluoroalkyl Substances: Analytical Techniques, Environmental Fate, and Health Effects. Int J Mol Sci. 2021;22.

[65] Li Y, Fletcher T, Mucs D, Scott K, Lindh CH, Tallving P, et al. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occup Environ Med. 2018;75:46-51.

* [66] The Agency for Toxic Substances and Disease Registry (ATSDR) USDoHaHS. Toxicological Profile for Perfluoroalkyls. 2018.

[67] Steenland K, Kugathasan S, Barr DB. PFOA and ulcerative colitis. Environ Res. 2018;165:317-21.

[68] Steenland K, Zhao L, Winquist A, Parks C. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. Environ Health Perspect. 2013;121:900-5.

[69] Stein CR, Savitz DA, Dougan M. Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. Am J Epidemiol. 2009;170:837-46.

[70] Wikstrom S, Lindh CH, Shu H, Bornehag CG. Early pregnancy serum levels of perfluoroalkyl substances and risk of preeclampsia in Swedish women. Sci Rep. 2019;9:9179.

[71] Huang R, Chen Q, Zhang L, Luo K, Chen L, Zhao S, et al. Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and the risk of hypertensive disorders of pregnancy. Environ Health. 2019;18:5.

* [72] Ait Bamai Y, Goudarzi H, Araki A, Okada E, Kashino I, Miyashita C, et al. Effect of prenatal exposure to per- and polyfluoroalkyl substances on childhood allergies and common infectious diseases in children up to age 7 years: The Hokkaido study on environment and children's health. Environ Int. 2020;143:105979.

[73] Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Budtz-Jorgensen E. Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds. Environ Health Perspect. 2017;125:077018.

[74] Stein CR, McGovern KJ, Pajak AM, Maglione PJ, Wolff MS. Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. Pediatr Res. 2016;79:348-57.

[75] Pennings JL, Jennen DG, Nygaard UC, Namork E, Haug LS, van Loveren H, et al. Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. J Immunotoxicol. 2016;13:173-80.

[76] Lenters V, Iszatt N, Forns J, Cechova E, Kocan A, Legler J, et al. Early-life exposure to persistent organic pollutants (OCPs, PBDEs, PCBs, PFASs) and attention-deficit/hyperactivity disorder: A multi-pollutant analysis of a Norwegian birth cohort. Environ Int. 2019;125:33-42.

[77] Vuong AM, Webster GM, Yolton K, Calafat AM, Muckle G, Lanphear BP, et al. Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) and neurobehavior in US children through 8 years of age: The HOME study. Environ Res. 2021;195:110825.

[78] Lien GW, Huang CC, Shiu JS, Chen MH, Hsieh WS, Guo YL, et al. Perfluoroalkyl substances in cord blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children. Chemosphere. 2016;156:118-27.

[79] Forns J, Verner MA, Iszatt N, Nowack N, Bach CC, Vrijheid M, et al. Early Life Exposure to Perfluoroalkyl Substances (PFAS) and ADHD: A Meta-Analysis of Nine European Population-Based Studies. Environ Health Perspect. 2020;128:57002.

[80] Skogheim TS, Villanger GD, Weyde KVF, Engel SM, Suren P, Oie MG, et al. Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children. Int J Hyg Environ Health. 2020;223:80-92.

[81] Quaak I, de Cock M, de Boer M, Lamoree M, Leonards P, van de Bor M. Prenatal Exposure to Perfluoroalkyl Substances and Behavioral Development in Children. Int J Environ Res Public Health. 2016;13.

[82] Liew Z, Ritz B, von Ehrenstein OS, Bech BH, Nohr EA, Fei C, et al. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort. Environ Health Perspect. 2015;123:367-73.

[83] Ge J, Wang C, Nie X, Yang J, Lu H, Song X, et al. ROS-mediated apoptosis of HAPI microglia through p53 signaling following PFOS exposure. Environ Toxicol Pharmacol. 2016;46:9-16.

[84] Wang C, Nie X, Zhang Y, Li T, Mao J, Liu X, et al. Reactive oxygen species mediate nitric oxide production through ERK/JNK MAPK signaling in HAPI microglia after PFOS exposure. Toxicol Appl Pharmacol. 2015;288:143-51.

[85] Yang J, Wang C, Nie X, Shi S, Xiao J, Ma X, et al. Perfluorooctane sulfonate mediates microglial activation and secretion of TNF-alpha through Ca(2)(+)-dependent PKC-NF-small ka, CyrillicB signaling. Int Immunopharmacol. 2015;28:52-60.

[86] Tukker AM, Bouwman LMS, van Kleef R, Hendriks HS, Legler J, Westerink RHS. Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) acutely affect human alpha1beta2gamma2L GABAA receptor and spontaneous neuronal network function in vitro. Sci Rep. 2020;10:5311.

[87] Braat S, Kooy RF. The GABAA Receptor as a Therapeutic Target for Neurodevelopmental Disorders. Neuron. 2015;86:1119-30.

* [88] Gaballah S, Swank A, Sobus JR, Howey XM, Schmid J, Catron T, et al. Evaluation of Developmental Toxicity, Developmental Neurotoxicity, and Tissue Dose in Zebrafish Exposed to GenX and Other PFAS. Environ Health Perspect. 2020;128:47005.

[89] Jantzen CE, Annunziato KA, Bugel SM, Cooper KR. PFOS, PFNA, and PFOA sub-lethal exposure to embryonic zebrafish have different toxicity profiles in terms of morphometrics, behavior and gene expression. Aquat Toxicol. 2016;175:160-70.

[90] Annunziato KM, Jantzen CE, Gronske MC, Cooper KR. Subtle morphometric, behavioral and gene expression effects in larval zebrafish exposed to PFHxA, PFHxS and 6:2 FTOH. Aquat Toxicol. 2019;208:126-37.

[91] Khezri A, Fraser TW, Nourizadeh-Lillabadi R, Kamstra JH, Berg V, Zimmer KE, et al. A Mixture of Persistent Organic Pollutants and Perfluorooctanesulfonic Acid Induces Similar Behavioural Responses, but Different Gene Expression Profiles in Zebrafish Larvae. Int J Mol Sci. 2017;18.

[92] Viberg H, Lee I, Eriksson P. Adult dose-dependent behavioral and cognitive disturbances after a single neonatal PFHxS dose. Toxicology. 2013;304:185-91.

[93] Menger F, Pohl J, Ahrens L, Carlsson G, Orn S. Behavioural effects and bioconcentration of per- and polyfluoroalkyl substances (PFASs) in zebrafish (Danio rerio) embryos. Chemosphere. 2020;245:125573.

[94] Reardon AJF, Karathra J, Ribbenstedt A, Benskin JP, MacDonald AM, Kinniburgh DW, et al. Neurodevelopmental and Metabolomic Responses from Prenatal Coexposure to Perfluorooctanesulfonate (PFOS) and Methylmercury (MeHg) in Sprague-Dawley Rats. Chem Res Toxicol. 2019;32:1656-69.

[95] Lyon GR, Fletcher JM, Barnes MC. Learning disabilities. Child psychopathology, 2nd ed. New York, NY, US: Guilford Press; 2003. p. 520-86.

[96] Siegel LS. IQ is irrelevant to the definition of learning disabilities. J Learn Disabil. 1989;22:469-78, 86.

[97] Souroulla AV, Panayiotou G, Kokkinos CM. The role of the teacher in identifying learning disabilities: a study using the McCarney Learning Disability Evaluation Scale (LDES). J Learn Disabil. 2009;42:483-93.

[98] Rudolph MD, Graham AM, Feczko E, Miranda-Dominguez O, Rasmussen JM, Nardos R, et al. Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. Nat Neurosci. 2018;21:765-72.

[99] Lorber M, Schecter A, Paepke O, Shropshire W, Christensen K, Birnbaum L. Exposure assessment of adult intake of bisphenol A (BPA) with emphasis on canned food dietary exposures. Environ Int. 2015;77:55-62.

[100] Rochester JR. Bisphenol A and human health: a review of the literature. Reprod Toxicol. 2013;42:132-55.

[101] Ferguson KK, Cantonwine DE, McElrath TF, Mukherjee B, Meeker JD. Corrigendum to "Repeated measures analysis of associations between urinary bisphenol-A concentrations and biomarkers of inflammation and oxidative stress in pregnancy" [Reprod. Toxicol. 66 (2016) 93-98]. Reprod Toxicol. 2019;90:166.

[102] Hong SB, Hong YC, Kim JW, Park EJ, Shin MS, Kim BN, et al. Bisphenol A in relation to behavior and learning of school-age children. J Child Psychol Psychiatry. 2013;54:890-9.

[103] Montevil M, Acevedo N, Schaeberle CM, Bharadwaj M, Fenton SE, Soto AM. A Combined Morphometric and Statistical Approach to Assess Nonmonotonicity in the Developing Mammary Gland of Rats in the CLARITY-BPA Study. Environ Health Perspect. 2020;128:57001.

[104] Mandrup K, Boberg J, Isling LK, Christiansen S, Hass U. Low-dose effects of bisphenol A on mammary gland development in rats. Andrology. 2016;4:673-83.

[105] Zhu J, Jiang L, Liu Y, Qian W, Liu J, Zhou J, et al. MAPK and NF-kappaB pathways are involved in bisphenol A-induced TNF-alpha and IL-6 production in BV2 microglial cells. Inflammation. 2015;38:637-48.

[106] Lu X, Li M, Wu C, Zhou C, Zhang J, Zhu Q, et al. Bisphenol A promotes macrophage proinflammatory subtype polarization via upregulation of IRF5 expression in vitro. Toxicol In Vitro. 2019;60:97-106.

[107] Gao L, Dong Y, Lin R, Meng Y, Wu F, Jia L. The imbalance of Treg/Th17 cells induced by perinatal bisphenol A exposure is associated with activation of the PI3K/Akt/mTOR signaling pathway in male offspring mice. Food Chem Toxicol. 2020;137:111177.

[108] Kaur S, Kinkade JA, Green MT, Martin RE, Willemse TE, Bivens NJ, et al. Disruption of global hypothalamic microRNA (miR) profiles and associated behavioral changes in California mice (Peromyscus californicus) developmentally exposed to endocrine disrupting chemicals. Horm Behav. 2021;128:104890.

[109] Morais LH, Schreiber HLt, Mazmanian SK. The gut microbiota-brain axis in behaviour and brain disorders. Nat Rev Microbiol. 2021;19:241-55.

[110] Feng D, Zhang H, Jiang X, Zou J, Li Q, Mai H, et al. Bisphenol A exposure induces gut microbiota dysbiosis and consequent activation of gut-liver axis leading to hepatic steatosis in CD-1 mice. Environ Pollut. 2020;265:114880.

[111] Chen L, Guo Y, Hu C, Lam PKS, Lam JCW, Zhou B. Dysbiosis of gut microbiota by chronic coexposure to titanium dioxide nanoparticles and bisphenol A: Implications for host health in zebrafish. Environ Pollut. 2018;234:307-17.

[112] Lai KP, Chung YT, Li R, Wan HT, Wong CK. Bisphenol A alters gut microbiome: Comparative metagenomics analysis. Environ Pollut. 2016;218:923-30.

* [113] Kaur S, Sarma SJ, Marshall BL, Liu Y, Kinkade JA, Bellamy MM, et al. Developmental exposure of California mice to endocrine disrupting chemicals and potential effects on the microbiome-gut-brain axis at adulthood. Sci Rep. 2020;10:10902.

[114] Catron TR, Keely SP, Brinkman NE, Zurlinden TJ, Wood CE, Wright JR, et al. Host Developmental Toxicity of BPA and BPA Alternatives Is Inversely Related to Microbiota Disruption in Zebrafish. Toxicological sciences : an official journal of the Society of Toxicology. 2019;167:468-83.

[115] Rutsch A, Kantsjo JB, Ronchi F. The Gut-Brain Axis: How Microbiota and Host Inflammasome Influence Brain Physiology and Pathology. Front Immunol. 2020;11:604179.

[116] Jasarevic E, Sieli PT, Twellman EE, Welsh TH, Jr., Schachtman TR, Roberts RM, et al. Disruption of adult expression of sexually selected traits by developmental exposure to bisphenol A. Proc Natl Acad Sci U S A. 2011;108:11715-20.

[117] Jasarevic E, Williams SA, Vandas GM, Ellersieck MR, Liao C, Kannan K, et al. Sex and dose-dependent effects of developmental exposure to bisphenol A on anxiety and spatial learning in deer mice (Peromyscus maniculatus bairdii) offspring. Horm Behav. 2013;63:180-9.

[118] Johnson SA, Javurek AB, Painter MS, Peritore MP, Ellersieck MR, Roberts RM, et al. Disruption of parenting behaviors in california mice, a monogamous rodent species, by endocrine disrupting chemicals. PLoS One. 2015;10:e0126284.

[119] Williams SA, Jasarevic E, Vandas GM, Warzak DA, Geary DC, Ellersieck MR, et al. Effects of developmental bisphenol A exposure on reproductive-related behaviors in California mice (Peromyscus californicus): a monogamous animal model. PLoS One. 2013;8:e55698.

[120] Society TE. What You Can do About EDCs. 2021.

[121] (USDA) USDoA. The National List of Allowed and Prohibited Substances. Electronic Code of Federal Regulations2021.

[122] Inguaggiatoa E. SG, Cioni G. Brain plasticity and early development: Implications for early intervention in neurodevelopmental disorders. Neuropsychiatrie de l'Enfance et de l'Adolescence.65:299-306.

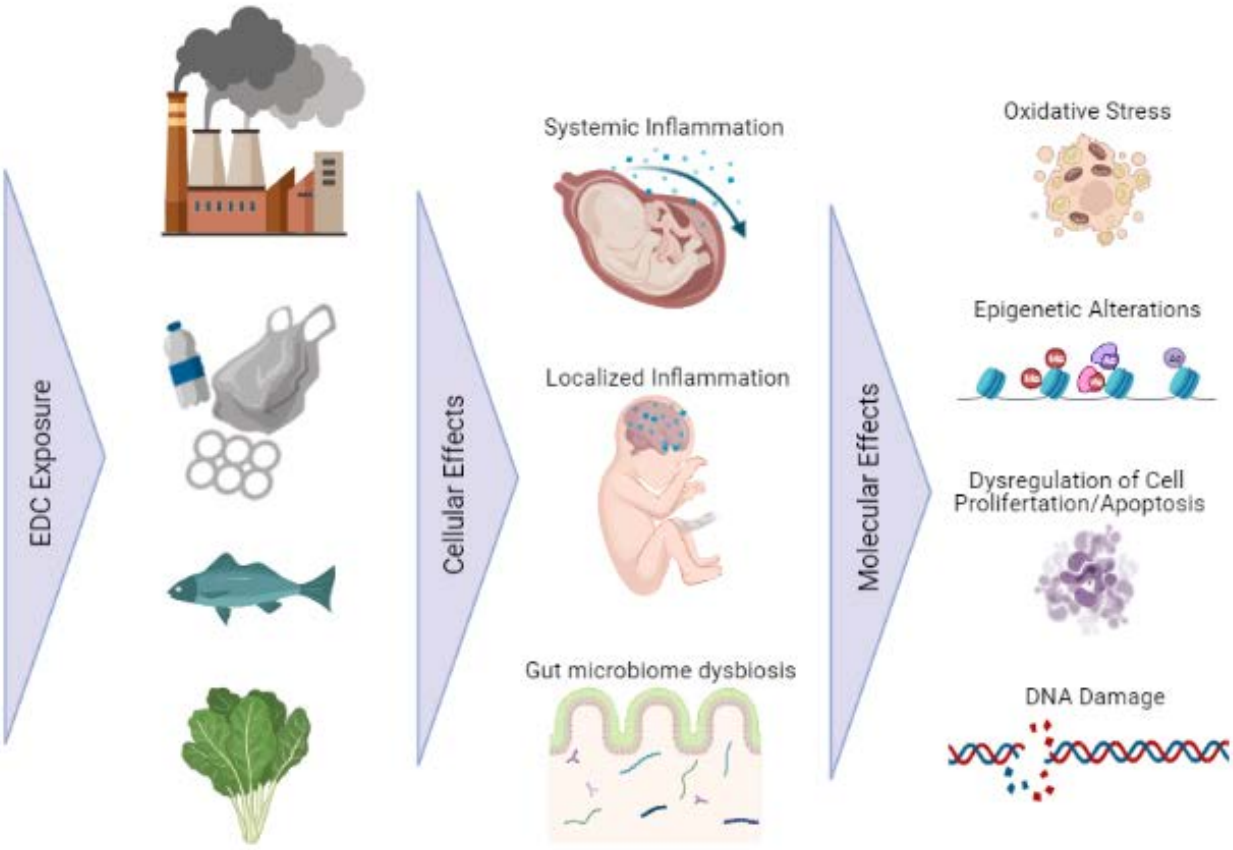

Figure 1. Exposure to some EDCs in the environment including in the air, household products, and on food, may be associated with cellular effects related to the immune system. This includes systemic inflammation like preeclampsia, localized inflammation in the brain tissue, or changes in the normal gut microbiome. In epidemiological and experimental studies, these observations are correlated to oxidative stress, epigenetic alterations, DNA damage, and changes in cell proliferation and death; if these molecular effects occur in the brain, they may affect how the brain develops and/or functions.

Chemical Class	Chemical Name	Species	Exposure administered or measured	Immune Effects	Neurodevelopmental Effect Reported	Citation
Phthalate	11 urinary metabolites measured, strongest association for MBP and MiBP	Human	Pregnant women	Pro-oxidative stress	Not measured	Ferguson K et al., 2014
Phthalate	9 urinary metabolites measured, strongest association for MBzP	Human	Pregnant women, one cohort samples collected at 16 and 26 weeks, the other during the 1st, 2nd, and 3rd trimesters.	Not measured	Increased ASD-like behavior in children as measured by Social Responsiveness Scale (SRS)	Patti et al., 2021
Phthalate	9 urinary metabolites measured and interpreted as a mixture exposure, strongest associations for maternal MBzP and MCPP observed.	Human	Pregnant women, two samples collected between weeks 6 and 21 (mean = 11) and between gestational weeks 26 and 42 (mean =33)	Not measured	Increased urinary phthalate metabolites in early pregnancy correlated to increased ASD as measured by SRS in both sexes. Increased phthalate exposure during late pregnancy correlated to increased externalizing behavior in boys as detected by the Behavioral Assessment System for Children (BASC-2)/	Day et al. 2021
Pthalate	14 urinary metabolites	Human	Pregnant women at high-risk for	Not measured	Most associations with both ASD and non-	Shin et al. 2018

			having a child with ASD, 2nd and 3rd trimester urine samples.		typical development (non-TD) were null, with the exception of MEP which was significantly associated with an increased risk of non-TD	
Pthalate	9 urinary metabolites	Human	Pregnant women 36-week urine samples, At age two years, the Bayley Scales of Infant and Toddler Development 3rd edition (BAYLEY-III)	79 small nucleotide polymorphisms (SNPs) associated with oxidative stress susceptibility.	Higher maternal phthalate levels in pregnancy were associated with subsequent offspring ASD and ASD traits. Multiple individual oxidative-stress related SNPs were associated with child neurodevelopment. Interaction was observed for continuous phthalate levels and several oxidative stress-related SNPs.	Ponsonby et al. 2020
Pthalate	DEHP	Human – ex vivo experiments	Peripheral blood mononuclear cells (PBMCs) and monocytes were obtained from autistic neurotypical (control) children. DEHP was administered (5 μM) to the	DEHP increased STAT3 expression/activity in blood mononuclear cells from ASD patients, but not in the control group. DEHP also enhanced inflammatory cytokines in monocytes of ASD children.	Children were diagnosed as ASD or neurotypical before the study.	Nadeem et al. 2020

			cultured monocytes and effects measured.			
Phthalates	DEHP	C57BL/6 and BTBR Mouse, male	5 mg/kg oral 2 weeks at the age of 6 weeks, analysis at the age of 8 weeks	DEHP was associated with oxidative stress and lack of antioxidant protection in peripheral innate immune cells (DCs, neutrophils) and cerebellum in BTBR mice. DEHP exposition led to lower extent of oxidative stress and more pronounced increase of antioxidative response in C57BL/6.	DEHP induced autism-like behavior (e.g., less interaction with novel mouse, increased repetitive behavior) in BTBR but not C57BL/6 mice.	Nadeem et al. 2021a
Phthalates	DEHP	C57BL/6 and BTBR Mouse, male	40 µg/kg via drinking water 3 weeks at the age of 3 weeks, analysis at the age of 6 and 12 weeks	DEHP triggered long-lasting epigenetic alterations (global DNA methylation in DCs, and tendentially in CD4+ T cells and cortex of BTBR mice and in DCs of C57BL/6 ↓). DEHP caused systemic and local inflammatory responses in BTBR and to some extent in C57BL/6 mice (i.e. % of TNF-α^+, MCP-1^+, and IL-6^+ expressing DCs ↑, plasma IL-6 levels ↑, expression of *TNF-α,*	DEHP induced autism-like behavior (e.g., less interaction with novel mouse, increased repetitive behavior) in BTBR and to a lower extent in C57BL/6 at 6 and 12 weeks of age.	Nadeem et al. 2021b

				MCP-1, and *IL-6* in DCs ↑, % of p-STAT3^{+} CD4^{+} T cells, IL-17A^{+} CD4^{+} T cells, *IL-17A* mRNA, and IL-17A protein levels in plasma ↑, cortical expression of *IL-6, TNF-α,* and *MCP-1* ↑).		
Phthalates	DEHP	ICR Mouse	10, 50, 200 mg/kg/d, oral GD7-PND21 analysis at the age of 6 (puberty) and 12 weeks of age (adulthood)	Not measured	DEHP induced anxious behavior (e.g. time spent in the open arms of elevated plus maze ↓) in pubertal mice (sex-independent) and in adult female mice. DEHP induced depression-like behavior (immobility time in forced swim task ↑) in pubertal and adult mice (both sexes). DEHP exposure led to abolition of certain sex-differences in behavior (e.g. frequency of grooming). Exposition to DEHP led to decreased activity of MAPK/ERKs signaling pathway and hippocampal levels of	Xu et al. 2015b

					ERβ and AR in (puberty and adult).	
Phthalates	DEHP	CD1 Mouse	200 µg, 500 mg, or 750 mg/kg/day, oral GD11-P analysis at the age of 16-22 month in males	Not measured	DEHP promoted anxious behaviors and impaired spatial and short-term recognition memory. DEHP exposition led to signs for neurodegeneration induced by oxidative stress and inflammation in hippocampal pyramidal neurons (i.e. neuron cell number ↓, *COX-2* expression ↑, DNA oxidation marker ↑, *AR* expression ↓). testosterone serum levels ↓	Barakat et al. 2018
Phthalate	DBP	C57BL/6 mice, male	10 or 50 mg/kg/d, intraperitoneal, 2 weeks	Not measured	DBP led to impaired hippocampal neurogenesis, spatial learning and memory (dose-dependent).	Lee et al. 2019
		murine pluripotent neural	100, 250, 500 µM for 72h		DPB promoted ROS generation and mitochondrial	

		progenitor cells			dysfunction but had no influence on apoptosis.	
Phthalate	phthalate mixture (DEP, DEHP, DBP, DiNP, DiBP, BBP)	Long-Evans Rat	200, or 1000 µg/kg/d, oral GD2-PND10	Not measured	Phthalate exposure led to sex-independent impairment of cognitive flexibility (attentional set-shift) and reduced neuron as well as synapse numbers and size of the mPFC.	Kougias et al. 2018b
Phthalates	phthalate mixture (DEP, DEHP, DBP, DiNP, DiBP, BBP)	Long-Evans Rat	200 or 1000 µg/kg, oral GD2-P10	Not measured	Phthalates impaired social behavior (i.e. time in playing ↓, passive contact ↑) especially in peri-adolescent males no effect on maternal behaviors	Kougias et al. 2018a
Phthalates	DBP	zebrafish *(Danio rerio)*	0.02, 0.2, 2 mM in water hpf6-72/96	DPB led to inhibition of neutrophil and macrophage development and impaired macrophage phagocytosis ability (concentration-dependent).	Not measured	Xu et al. 2015a

Phthalates	DBP, DINP, BBP	zebrafish *(Danio rerio)*	1.25, 2.5, 5, 10, and 20 μM, hpf2-dpf3	Not measured	Phthalate exposure disturbed the expression of estrogen receptors (*esr1, esr2a, esr2b*), and led to impaired neurogenesis in the brain during embryonic development (i.e. brain size and number of proliferating neurons ↓)	Xu et al. 2020
		neurons, human	1 nm for 30 min		Phthalates caused double-strand DNA breaks.	
Phthalates	DEHP	THP-1 cell line (murine macrophage-like cell line)	200 μM for 3 h	DEHP promoted an inflammatory phenotype (i.e. TNF-α, IL-1β, IL-8, and IL-6 secretion in culture supernatant ↑, expression of *IL-8, CXCL1, CXCL2, CXCL3, CXCL6, CCL3, MMP3, MMP10, MMP14, CSF2, TNF-α, IL-1β,* and *IL-6* ↑, NF-κB activation).	Not measured	Nishioka et al. 2012
Phthalates	MEHP, BBP, BPA, DEHP, PFOA, PFOS	RAW 264.7 cell line (murine	5 and 50 μM for 24 h	MEHP promoted inflammatory responses.	Not measured	Park et al. 2019

		macrophage cell line)		MEHP led to decreased SIRT protein expression and activity. MEHP induced oxidative stress. Exposure to MEHP led to enhanced acetylation of NF-κB , activated NLRP3 inflammasome and induced secretion of IL-1β.		
Phthalate	DBP	primary cortical neurons, murine	10 nM-100 µM for 3-48 h	Not measured	DBP induced apoptosis (ROS, caspase-3 and LDH activities ↑). DBP interferes with AhR, Erα and PPARγ pathways (i.e. *Erα* and *PPARγ* expression ↓, *Ahr* expression ↑).	Wójtowicz et al. 2017

Table 1. Literature reviewed investigating how phthalates may affect the immune system during pregnancy or childhood and/or neurodevelopmental effects reminiscent of autism. Acronyms defined as follows, MBP=monobutyl phthalate, MiBP=diisobutyl phthalate, MBzP=monobenzyl phthalate, MCPP=mono-(3-carboxypropyl) phthalate, MEHP=mono-(2-ethylhexyl) phthalate, BBP=benzyl butyl pthalate, BBzP=benzyl butyl pthalate, DBP=dibutyl pthalate, DiNP=di-isononyl pthalate, DEHP=bis(2-ethylhexyl) pthalate, DEP=diethyl pthalate, DHpP=diheptyl pthalate, DiBP=di-isobutyl pthalate, DIDP=diisodecyl pthalate, DMP=dimethyl pthalate, and DnOP=di-n-octyl pthalate.

Chemical Class	Chemical Name	Species	Exposure administered or measured	Immune Effects	Neurodevelopmental Effect Reported	Citation
PFAS	PFOA, PFOS	Human	Self-reported pregnancy complications and birth defect incidence which were linked to serum chemical concentrations.	Modest associations were detected linking levels of PFOA and PFOS with preeclampsia.	None, but PFOA was weakly linked with birth defects and PFOS with low birth weight.	Sten et al. 2009
PFAS	8 PFAS were quantified in serum: PFOS, PFOA, PFHxS, PFNA, PFDA, PFUnDA, PFHpA, and PFDoDA	Human	PFAS were measured at median 10 gestational weeks and cases of preeclampsia were postnatally identified from registers.	PFOS and PFNA were associated with an increased incidence of preeclampsia.	Not measured	Wikstrom et al. 2019
PFAS	PFOA, PFOS, PFNA, PFUA, PFDA, PFHxS, PFDoA, PFBS	Human	Cord blood PFAS concentrations were then correlated to presence of pregnancy hypertension.	PFBS was positively associated with preeclampsia in a dose-response pattern.	Not measured.	Huang et al. 2019
PFAS	PFOA, PFOS, PFNA,	Human	Maternal blood PFAS concentrations at	Exposure to PFOS, PFNA, PFUnDA, PFDoDA, and PFTrDA were associated with	Not measured	Bamai et al. 2020

Chemical Class	Chemical Name	Species	Exposure administered or measured	Immune Effects	Neurodevelopmental Effect Reported	Citation
	PFDA, PFHxS, PFDoDA, PFUnDA, PFTrDA		28-32 weeks of pregnancy were correlated to incidence of allergies and infectious diseases in the resulting children.	reduced risks of wheeze, eczema, and rhino-conjunctivitis, and with increased risks of pneumonia and RSV infection. This inverse association of allergic symptoms and increased risks of infectious diseases suggests immunosuppressive effects.		
PFAS	PFHxS, PFOA, PFOS, PFNA, PFDA	Human	Children serum PFAS concentrations were correlated to antibody titter concentrations a 7 and 13 years of age.	Diphtheria antibody concentrations decreased at elevated PFAS concentrations; the associations were statistically significant for PFDA and PFOA at 13 years. Modeling showed that a doubling in PFAS exposure at 7 years was associated with losses in diphtheria antibody concentrations at 13 years of 10–30% for the five PFAS measured.	Not measured	Grandjean et al. 2017
PFAS	PFOS, PFOA, PFHxS, PFNA	Human	In 12-19 year, old children, PFAS serum concentrations were examined in relation to measles, mumps, and rubella antibody titers and to	PFOS was linked to a decrease in rubella antibody concentration and a decrease in mumps antibody titers. Children with higher PFOS concentration were less likely to be sensitized to any allergen.	Not measured	Sten et al. 2016

Chemical Class	Chemical Name	Species	Exposure administered or measured	Immune Effects	Neurodevelopmental Effect Reported	Citation
			presence of allergic conditions and allergic sensitization.			
PFAS	19 PFAS were measured in maternal serum, but only PFOS, PFOA, PFNA and PFHxS correlated to health effects.	Humans	Transcriptomics profiles in neonatal cord blood and their association with maternal PFAS exposure, anti-rubella antibody levels at 3 years of age and the number of common cold episodes until 3 years	Analyses identified a profile of 52 PFAS exposure-associated genes in cord blood that were in common with genes associated with rubella titers and/or common cold episodes. This includes several immunomodulatory genes (CYTL1, IL27) as well as other immune-associated genes.	Not measured	Pennings et al. 2015
PFAS	27 persistent organic pollutants were measured in breast milk, including PFOA and PFOS	Human	Associations between early-life exposure to 27 contaminants in breast milk and doctor-diagnosed ADHD in school-age children.	Not measured	Odds of ADHD increased with increasing PFOS levels, with higher odds in girls than boys.	Lenters et al. 2019
PFAS	PFOS, PFHxS, PFOA, and PFNA	Human	Maternal PFAS were measured in serum at least once during pregnancy	Not measured	PFOS, PFHxS, and PFNA were associated with higher Behavioral Assessment System for Children 2 scores and	Vuong et al. 2021

Chemical Class	Chemical Name	Species	Exposure administered or measured	Immune Effects	Neurodevelopmental Effect Reported	Citation
			or at delivery, and then correlated to child behavior using various metrics at 5 and 8 years of age.		increased odds of externalizing behaviors, including hyperactivity. PFHxS was also associated with internalizing problems and somatization. PFOS and PFNA were significantly associated with DISC-YC symptoms and diagnostic criteria related to hyperactive-impulsive type ADHD. Prenatal PFNA was associated with increased risk of any-type ADHD.	
PFAS	PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFBS, PFHxS, PFOS, 6:2 FTSA + mixture	zebrafish *(Danio rerio)*	0,0001-100 mg/L (0,0002-400 µM) in water hpf0-144	Not measured	PFASs alone and mixture influenced swimming behavior, however mixture induced effect was less than that of the individual PFAS.	Menger et al. 2020
PFAS	ADONA, GenX Free Acid PFESA1, PFHxA, PFHxS,	zebrafish *(Danio rerio)*	4.4-80.0 µM ADONA, PFESA1, PFHxA, PFHxS, or PFOA, 0.2-3.1 µM PFOS dpf0-5	Not measured	Individual PFASs have different effects on development and behavior. PFOS and PFHxS elicited adverse effects on development (i.e. failed swim bladder inflation, abnormal ventroflexion of the	(Gaballah et al. 2020)

Chemical Class	Chemical Name	Species	Exposure administered or measured	Immune Effects	Neurodevelopmental Effect Reported	Citation
	PFOA, PFOS				tail) and were associated with and hyperactivity.	
PFAS	PFHxA, PFHxS, 6:2 FTOH	zebrafish *(Danio rerio)*	0.02-20 µM hpf3-120	PFAS induced *tgfb1a, bdnf,* and *ap1s1* expression.	PFHxA decreased and FTOH increased activity (e.g. swim distance, velocity, crosses through the center of the arena).	(Annunziato et al. 2019)
PFAS	PFOS, PFOA, PFDA, PFNA, PFHxS, PFUnDA + mixture	zebrafish *(Danio rerio)*	5,48 µM (100 x human serum level) alone or in mixtures hpf6-96	Not measured	PFAS mixture and PFOS alone increased swimming speed (exposure at hpf48-96 but not hpf6-48) and altered expression of genes related to neurodevelopment.	(Khezri et al. 2017)
PFAS	PFOS, PFOA, PFNA	zebrafish *(Danio rerio)*	0.02, 0.2, 2.0 µM dpf0-5	Not measured	All PFAs induced hyperactive activity (e.g. swim distance, velocity, crosses through the center of the arena; dpf14), morphological changes and altered expression of genes related to behavior.	(Jantzen et al. 2016)
PFAS	PFHxS	NMRI mouse	0.61, 6.1 or 9.2 mg PFHxS/kg body weight single, oral application at PND10	Not measured	A single neonatal exposure caused spontaneous behavioral effects in a novel environment in 2- and 4-months old mice.	(Viberg et al. 2013)

Chemical Class	Chemical Name	Species	Exposure administered or measured	Immune Effects	Neurodevelopmental Effect Reported	Citation
PFAS	PFOS and MeHg	Sprague Dawley Rat	1 mg/kg BW PFOS, 1 mg/kg BW MeHg, 0.1 mg/kg PFOS + 1 mg/kg MeHg, or of 1 mg/kg PFOS + 1 mg/kg MeHg GD1-PND21	Not measured	PFOS and MeHg alone, but not in combination caused hyperactivity. Mixture, but not PFOS and MeHg alone led to anxiety-related behavior. PFOS and MeHg alone, but not in combination led to altered cortical metabolite concentrations (e.g. GABA and amino acids ↑).	(Reardon et al. 2019)
PFAS	PFOS	HAPI microglia cell line, rat	1, 10, 100, and 200 µM for 24h	Not measured	PFOS induced apoptosis (caspase-3 and PARP ↑) by oxidative stress and thus p53 expression.	(Ge et al. 2016)
PFAS	PFOS, PFOA	$\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptor expressing *Xenopus laevis* oocytes primary cortical neurons, rat hiPSC-derived neurons	0.01-100 µM	Not measured	PFAS act as non-competitive inhibitors of the $GABA_A$ receptor (0.1 µM PFOS, 1 µM PFOA), but had different effects on network activity in rat primary cortical cultures (100 µM PFOS ↑) and human hiPSC neurons (PFOS and PFOA ↓).	(Tukker et al. 2020)

Table 2. Literature reviewed investigating how per- and polyfluoroalkyl substances (PFAS) may affect the immune system during pregnancy or childhood and/or neurodevelopmental effects reminiscent of attention deficit hyperactivity disorder. Acronyms defined as follows, PFOA=perfluorooctanoic acid, PFOS=perfluorooctanesulfonic acid, PFHxS=perfluorohexane sulfonate, PFNA= perfluorononanoic acid, PFBS=perfluorobutanesulfonic acid, PFHxA= perfluorohexanoic acid, PFDA= perfluorodecanoic acid, PFUnDA=perfluoroundecanoic acid, PFHpA=perfluoroheptanoic acid, PFDoDA=perfluorododecanoic acid, GenX/HFPO-DA=hexafluoropropylene oxide dimer acid, 6:2 FTOH=6:2 fluorotelomer alcohol, 6:2 FTSA=6:2 fluorotelomersulfonate, 8:8 PFPiA=8:8 perfluoroalkyl phosphinic acid, ADONA=4,8-dioxa-3H-perfluorononanoate, PFESA1=perfluoro-3,6-dioxa-4-methyl-7-octene-1-sulfonic acid, PFHpA=perfluoroheptanoate, PFHpS=perfluoroheptanesulfonic acid, PFPeS=perfluoropentanesulfonic acid, PFUnDA=perfluoroundecanoic acid, and hiPSC=human induced pluripotent stem cells.

Chemical Class	Chemical Name	Species	Exposure administered or measured	Immune Effects	Neurodevelopmental Effect Reported	Citation
Bisphenol	BPA	Human	Pregnant women (10-35 weeks)	Pro-inflammatory and pro-oxidative stress	Not measured	Ferguson K et al., 2016
Bisphenol	BPA	Human	Children, ages 8-11	Not measured	Abnormal listening as measured by Learning Disability Evaluation Scale (LDES)	Hong S et al., 2013
Bisphenol	BPA	Deer mouse (*Peromyscus maniculatus bairdii*)	50 mg, 5 mg, 50 µg/kg feed weight two weeks prior to breeding, throughout gestation and lactation	Not measured	BPA induced dose-dependent impairments in spatial learning, anxiety and decreased exploratory behaviors in males but not females (two upper but not the lowest dose).	(Jašarević et al. 2013)
Bisphenol	BPA	Deer mouse (*Peromyscus maniculatus bairdii*)	50 mg/kg feed weight two weeks prior to breeding, throughout	Not measured	BPA led to impairment of spatial learning abilities and exploratory behaviors in male offspring.	(Jašarević et al. 2011)

			gestation and lactation		Female mice preferred control males to BPA-exposed males.	
Bisphenol	BPA	California mice *(Peromyscus californicus)*	5 mg/kg feed weight, low dose-LD or 50 mg/kg, upper dose-UD additionally: genistein (250 mg/kg feed weight) two weeks prior to breeding, throughout gestation and lactation analysis at 90 days of age	BPA had no effect on α- and β-diversity of fecal microbiota, however certain taxa were influenced in a sex-dependent manner.	BPA promoted stereotypical/repetitive behaviors, but had no effect on other autistic-like behaviors (social behavior, spatial learning and memory, communication behavior). There was a correlation between bacteria, metabolites and behavior.	(Kaur et al. 2020)
Bisphenol	BPA	California mice *(Peromyscus californicus)*	50 mg/kg feed weight two weeks prior to breeding, throughout gestation and lactation	Not measured	F1 females have impaired parental care behavior (time nursing, grooming and being associated with their pups ↓) than controls, while care of pups by F1 males was less affected. Female mice might sense a male partner previously exposed, which lead to a lack of acceptance and	(Johnson et al. 2015)

					reduced parental care behavior. There was little effect on F2 weight gain.	
Bisphenol	BPA	California mice *(Peromyscus californicus)*	50 mg/kg feed weight two weeks prior to breeding, throughout gestation and lactation	Not measured	BPA had no effect on spatial navigational skills in either males or females, but abolished sex difference in exploratory behavior (less urinary marking behavior when confronting an unfamiliar male).	(Williams et al. 2013)
Bisphenol	BPA	California mice *(Peromyscus californicus)*	5 or 50 mg/kg feed weight two weeks prior to breeding, throughout gestation and lactation	BPA exposition resulted in sex-dependent alterations in global hypothalamic miR/small RNA expression (e.g. miR146a that might be involved in pathways such as response to nerve growth factor, regulation of innate immune response, positive regulation of defense response)	BPA had only mild effects on spatial learning and memory, however social communication behaviors to a novel individual was impaired.	(Kaur et al. 2021)
Bisphenol	BPA	C57BL/6 mice	250 ng/kg/d, subcutaneous GD10-PND20	Not measured	BPA induced anxiety-like behavior. Exposure to BPA affected the dopamine	(Matsuda et al. 2012)

					pathway in the brain (e.g. DA levels ↑ and DOPAC/DA ratio ↓) in male, but not in female mice.	
Bisphenol	BPA	Long-Evans Rat	0, 40, or 400 µg/kg/d, oral mother: GD2-P pups: PND1-10	BPA exposure increased secretion of cytokines (TNF-α, MCP-1, VEGF) in mPFC of male, but not female offspring. BPA had long-lasting effect on expression of *Esr1* in the mPFC of females.	BPA administration was associated with impaired social behavior (time in playing ↓, time alone and in passive contact ↑) in peri-adolescent mice. BPA had a dose-dependent anxiolytic effect in males, but not in females.	(Wise et al. 2019)
Bisphenol	BPA, BPAF, BPB, BPF, BPS	zebrafish *(Danio rerio)*	0.0-45.0 µM in water dpf 1-10	BPA, BPS and BPF elicited concentration-dependent shift of microbiota and certain pathways.	None of the BP analogues had behavioral effects.	(Catron et al. 2019)
Bisphenol	BPA	zebrafish *(Danio rerio)*	100 µg/L titanium dioxide nanoparticles; 0, 2, and 20 µg/L BPA or their binary mixtures in water 3 months	Mixture and individual substances caused dysbiosis (alterations at phyla level, loss of diversity) in a sex- and dose-dependent manner. IL-1β secretion after exposure to BPA alone or in mixture	Intestinal serotonin levels were affected in a sex-dependent manner. There was a correlation between bacteria and physiological	(Chen et al. 2018)

				was decreased in male's intestine but elevated in the female gut. BPA alone and the mixture induce oxidative stress in the gut in a sex-dependent manner.	parameters (e.g. Pseudomonas-ROS).	
Bisphenol	BPA	BV2 cell line (murine microglial cell line)	10, 100, and 1000 nM		BPA led to BV2 cells activation with increase in TNF-α and IL-6 expression, partially mediated via ER and NF-κB activation.	(Zhu et al. 2015)
Bisphenol	BPA	T cells (murine and human)	0.05 to 50,000 nM for 72 h-5 d	BPA exposition induced IL-17 production in mouse but nut human T cells (0.05 nM), in an AhR-dependent manner.	Not measured	(Malaisé et al. 2020)

Table 3. Literature reviewed investigating how bisphenols may affect the immune system during pregnancy or childhood and/or neurodevelopmental effects reminiscent of learning disabilities. Acronyms defined as follows, BPA = bisphenol A, BPS = bisphenol S, BPAF= bisphenol AF, and BPF= bisphenol F.