Elucidating the Links Between Endocrine Disruptors and Neurodevelopment

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Recent data indicate that approximately 12% of children in the United States are affected by neurodevelopmental disorders, including attention deficit hyperactivity disorder, learning disorders, intellectual disabilities, and autism spectrum disorders. Accumulating evidence indicates a multifactorial etiology for these disorders, with social, physical, genetic susceptibility, nutritional factors, and chemical toxicants acting together to influence risk. Exposure to endocrine-disrupting chemicals during the early stages of life can disrupt normal patterns of development and thus alter brain function and disease susceptibility later in life. This article highlights research efforts and pinpoints approaches that could shed light on the possible associations between environmental chemicals that act on the endocrine system and compromised neurodevelopmental outcomes. (*Endocrinology* 156: 1941–1951, 2015)

Endocrine-disrupting chemicals (EDCs) are a broad group of compounds that when ingested or absorbed can mimic, block, or otherwise alter the activity of hormones, thereby disrupting normal growth and development. EDCs include a number of wide-ranging chemical substances, both natural and manmade, such as pharmaceuticals, industrial by-products (dioxin and dioxin-like compounds), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and other pesticides, herbicides, and fungicides, components of plastics such as bisphenol A (BPA) and phthalates, solvents, surface protectors, flame retardants, and naturally occurring chemicals such as phytoestrogens (which originate in plants). EDCs are ubiquitous in our environment, food, and consumer products, making exposure to these toxicants common and widespread in nonoccupational settings (1). Exposure to many chemicals including EDCs during early stages of development can disrupt normal patterns of development and thus interfere with the body's endocrine system and produce adverse neurological, reproductive, cardiovascular, metabolic, and immune effects in humans. Some studies have shown that these alterations can be transmitted across generations (2).

Classically, "endocrine disruptors" have been defined as chemicals or chemical mixtures that exert their actions by modifying the actions of estrogens, androgens, and thyroid hormones. These changes can alter normal hormone levels, inhibit or stimulate the production of hormones, or change the way hormones travel through the body, thus affecting the functions that these hormones control. However, recent studies show that the mechanisms by which EDCs act are much broader than previously recognized. In addition to altering nuclear receptor signaling, EDCs are capable of acting through membrane receptors, nonsteroid receptors, and transcriptional coactivators, as well as in enzymatic pathways involved in steroid biosynthesis and/or metabolism and through numerous other mechanisms that converge upon endocrine and reproductive systems. Other less well-known mechanisms of action of

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Abbreviations: ADHD, attention deficit hyperactivity disorder; AhR, aryl hydrocarbon receptor; ASD, autism spectrum disorder; BaP, benzo[a]pyrene; BPA, bisphenol A; CPF, chlorpyrifos; DDT, dichlorodiphenyltrichloroethane; ED, endocrine-disrupting; EDC, endocrine-disrupting chemical; HOME, Health Outcomes and Measures of the Environment; HPG, hypothalamic-pituitary-gonadal; LMW, low molecular weight; PAH, polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyl.

EDCs include effects on cell signaling and trafficking (3) and alterations in epigenetic programming (4). Thus, the wide-ranging actions of EDCs may result in subtle disruptions in development or they may have more severe effects. This evidence is reflected in the broad World Health Organization definition of EDCs: "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations" (5).

Steroid hormones have significant effects across the nervous system and play a critical role in brain organization of the neuroendocrine circuitry that coordinates sex-specific physiology and behavior in vertebrates. EDCs can influence sex-specific development and behaviors through their direct effects on the hypothalamic-pituitary-gonadal (HPG) axis, which coordinates reproductive maturation by release of GnRH (6), and the hypothalamic-pituitary-adrenal axis, which mediates stress response. The developing brain contains receptors for steroid hormones, which play a role in neural cell migration, differentiation, synaptogenesis, and myelination, which coordinates sex-specific physiology and behavior in vertebrates (Figure 1, A and B). Through their interaction with hormone/receptor complexes, EDCs can alter any of these developmental processes (7). For example, environmental estrogens have been shown to accelerate neurogenesis and neuronal migration in the developing hypothalamus, the region of the brain that plays a particularly important role in feeding behaviors (8, 9). Improper programming of hypothalamic neural populations, specifically the alteration of the activity of appetite and satiety neurons, may result in modified eating habits in adolescents and adults (10).

EDCs have been shown to exert neurotoxic effects that are complex and lead to subtle impairments that are independent of, or indirectly related to, their effects on hormones (11). For instance, EDCs can disrupt the synthesis, transport, and release of many neurotransmitters, including (Figure 1C) dopamine, serotonin, norepinephrine, and glutamate, which play key roles in modulating behavior, cognition, learning, and memory (12). In addition, many neurons coexpress steroid hormone receptors during different stages of development, making them likely targets of EDCs (11, 13). Therefore, EDCs impinging on steroid-sensitive circuitry in the brain can exert effects on cognition, learning, memory, and other nonreproductive behaviors, such as metabolism, as well as reproductive neuroendocrine systems.

Determining whether a neurotoxicant should also be categorized as an EDC is not always clear if the mechanism of action is not well established. For instance, heavy metals such as lead, cadmium, mercury, arsenic, manganese, and zinc are known neurotoxicants, yet are not commonly classified as EDCs. Nonetheless, these metals have been reported in a substantial number of studies to have endocrine-disrupting (ED) properties (14). Studies have dem-

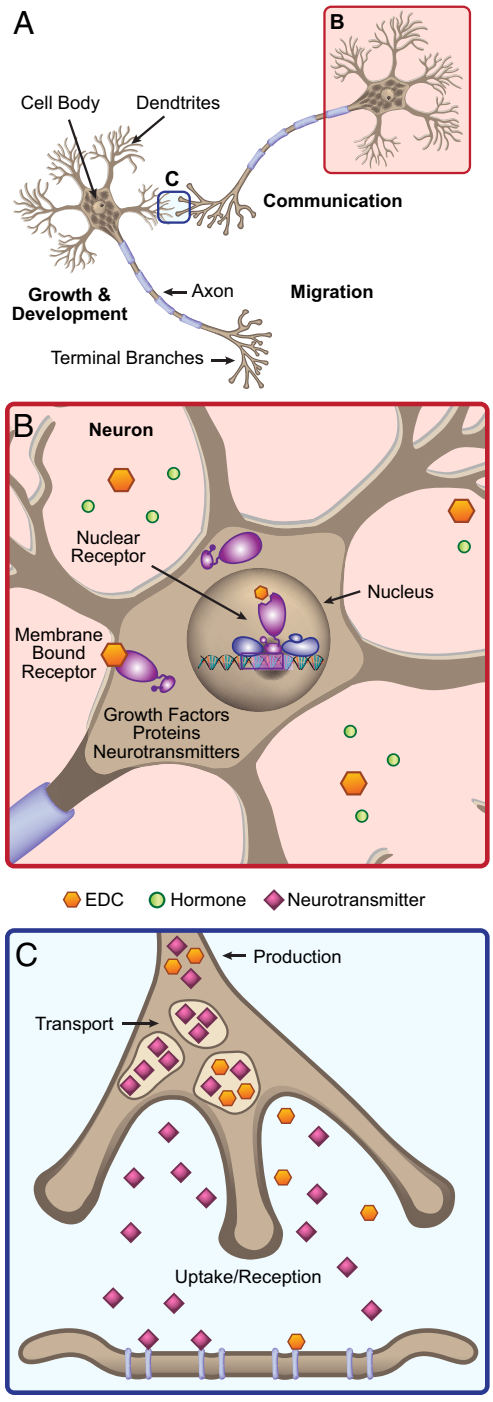

Figure 1. Actions of EDCs on neural cells. A, EDC interference can alter neuron-to-neuron communication, neuronal growth and development, and migration patterns. B, EDCs can act as hormone mimics by binding to and activating hormone receptors throughout the cell, resulting in changes in both genomic and nongenomic cellular responses. C, In addition to having hormone-specific receptors on membranes, in the cytoplasm, and in the nucleus, neurons have neurotransmitter-specific receptors in the synaptic region between neurons where electrical and chemical impulses travel.

onstrated that inorganic metals can interact with the steroid-binding domain of certain nuclear receptors (15) or bind to zinc finger regions of receptors and thus alter their interaction with DNA and subsequent transcriptional activity (16). Evidence also suggests that mixtures of chemicals, including both traditional EDCs and non-EDCs, may exhibit additive and/or synergistic neurotoxic effects. Although these data are provocative, the link between the ED properties of metals and neurodevelopment is still unclear and needs further study (14). The elucidation of this relationship could provide causal information about the importance of the timing, duration, and pattern of exposures to neurodevelopment. Indeed, the fetal period and early childhood years have been shown to be particularly susceptible times in development of harmful effects from environmental toxicants, many of which are classified as EDCs (Table 1) (13, 17–21). In addition, although it is well known that neurological conditions are sexually dimorphic and that females and males respond differently to hormones, the clarification of the mechanisms that result in these effects is necessary to draw further conclusions about sex-based differences. The purpose of this review is to highlight emerging evidence on a select group of EDCs that influence neurodevelopment and identify key research gaps and needs to effectively move the field forward.

Review of EDCs Linked to Neurotoxicity

PCBs

Some of the most compelling evidence for the link between EDC exposure and impaired cognitive function involves PCBs, chemicals that were widely used as coolants, plasticizers, and flame retardants, among many other uses, until their production was halted in the United States in 1979. PCBs are mixtures of up to 209 different compounds, and depending on the congener's specific structure, may act as estrogens or dioxins. Despite the ban, PCBs continue to exist ubiquitously in wildlife and humans because of their resistance to degradation and accumulation in fatty tissues (22). Negative associations between prenatal PCB exposure and measures of cognitive function in infancy or childhood, learning, memory, and IQ have been found in studies from Taiwan, Michigan, New York, The Netherlands, Germany, and the Faroe Islands (23). The Netherlands and Michigan cohort studies also reported associations between PCB exposures and problems such as inattention, impulsiveness, and other attention deficit hyperactivity disorder (ADHD)–related behaviors (24, 25). In addition to the cognitive and ADHD-like effects observed, the children from Taiwan also exhibited sexually dimorphic behavior with boys displaying a deficit in spatial abilities (26).

Although the precise mechanisms by which PCBs alter human brain functions are not well understood, studies have shown that they interact with nuclear estrogen receptors in sexually dimorphic regions of the brain. For example, PCB exposure affects neuronal connectivity in hippocampal neurons (27) and hypothalamic neurons (27). Altered neuronal connectivity is a common feature of many neurobehavioral conditions, including ADHD and autism spectrum disorders (ASDs). In rodents, PCB exposure causes accelerated female puberty, irregular estrous cycles, and premature anestrus. These effects could result

Table 1. Environmental Chemicals Associated With Impaired Neurodevelopment Outcomes

Chemical	Commercial Use	Neurodevelopmental Effects	Potential Mechanism	References
PCBs	Coolants, plasticizers, and flame retardants	Impaired learning, memory, and IQ and behavioral deficits such as inattention and impulsiveness	Altered neuronal connectivity; thyroid hormone disruption	20–28
PAHs	Result from incomplete fossil fuel combustion	Lower mental development index, negative associations with IQ, adverse effects on fetal growth, cognitive development, and behavioral disorders	AhR cross talk and activation	29–42
Phthalates	Plasticizers, adhesives, personal care products	Impaired concentration in girls; diminished motor function and masculine behavior in boys	Inhibition of testosterone synthesis	43–50
BPA	Plastics and epoxy resins	Aggression, hyperactivity, anxiety, social behavior	Estrogenic effects, nonspecific signaling pathways	54–60
Metals	Many commercial applications	Neurodevelopment, cognition, impaired cortisol metabolism	Alteration of sex hormone levels, nondirect effects	14, 61–71
Pesticides	Pest deterrent, biocide	Lower mental developmental index, attention deficits, reduced childhood IQ	Alteration of inhibition/excitation, disruption of thyroid hormones, iodine uptake disruption	72–87

from interference anywhere along the HPG axis during prenatal and early postnatal development (28). PCB exposures have also been shown to induce effects on hypothalamic sexual differentiation and neuroendocrine gene and protein expression in adulthood (28).

Studies in rodents show that PCB exposures decrease serum thyroid hormone levels and produce thyromimetic effects on brain development similar to those observed in PCB-exposed human populations (29). Further, it has been shown that PCB exposure alters serotonin and 5-hydroxyindoleacetic acid concentrations in the brains of several tested animal species. Mechanisms through which PCB has been suggested to affect serotonin concentrations include binding to aryl hydrocarbon receptors (AhRs) to inhibit the synthesizing enzyme tryptophan hydrolyase (dioxin-like PCBs) and binding to vesicular monoamine transporter 2 to inhibit serotonin uptake into vesicles (nondioxin-like PCBs) (30). PCB exposure and its association with compromised cognitive function provides a solid basis for the understanding of the relationship between EDC exposure and neurodevelopment.

PAHs

PAHs comprise another broad class of airborne compounds that appear to have neuroendocrine effects. PAHs are ubiquitous in urban environments, primarily as a result of incomplete fossil fuel combustion of carbon-containing matter, such as wood, coal, and tobacco. PAHs can also be found in crude oil and coal tar emissions. Certain PAHs mimic steroid hormones and can accumulate in adipose tissue and cross the placenta and fetal blood-brain barrier (31). In one of the first studies to look at the effects of prenatal exposure to airborne PAHs on early child development, researchers evaluated child mental and psychomotor development from prenatal PAH exposure measured by personal air sampling during pregnancy in a cohort of nonsmoking African-American and Dominican mothers and children in New York. Individuals in the upper quartile of PAH exposure had a lower mental development index at age 3 and had greater odds of cognitive developmental delay (32). Intelligence assessments in children from this cohort at 5 years of age found negative associations between high PAH level exposures and both full-scale and verbal IQ, with high-exposure individuals scoring 4.31 and 4.67 points lower, respectively, than those with lower PAH exposures (32). A parallel cohort study of Polish Caucasians reported adverse effects of exposure on fetal growth and cognitive development (33, 34).

Studies using animals exposed to PAHs during the prenatal and neonatal periods have reported neurodevelopmental and behavioral effects including depression-like symptoms and memory impairment in the absence of other overt toxicological effects (35, 36). Other studies have shown that PAH exposures have an impact on dopaminergic activity and gene expression patterns in the brain (37, 38). Although the mechanisms of action have not been fully elucidated, numerous studies report that PAHs act as endocrine disruptors through their ability to bind to and activate the AhR and induce monooxygenases (39). In addition, some PAHs have been found to exhibit ED effects, such as estrogenic, antiandrogenic, and antiestrogenic activity, partly through crosstalk between AhR and estrogen receptor signaling (40). The PAH benzo[a]pyrene (BaP), a carcinogen found in coal tar and automobile exhaust and produced by wood-burning fires, exhibits many toxic properties. BaP has been reported to alter the levels of noradrenaline, dopamine, and serotonin and/or their metabolites in certain brain regions, suggesting that chemical disruption of neural synaptic receptors may underlie the anomalies in learning and cognitive development associated with PAH exposure (31, 41). In mice, metabolites of BaPs produced during periods of postnatal synaptic development (42) have been shown to disrupt expression of important neurodevelopment genes, specifically those that mediate the function of tyrosine kinase receptors such as platelet-derived growth factor receptor α, epidermal growth factor receptor, and the MET receptor tyrosine kinase (43). In the brain, these receptors facilitate synaptic plasticity and hippocampus-dependent learning and memory. Taken together, these mechanistic studies suggest that early life PAH exposures could play an important role in neurodevelopment disorders such as ASD and ADHD (44).

Plastics: phthalates and BPA

Two broad groups of EDCs that have been implicated as neurotoxicants include phthalates and BPA. Phthalates are industrial plasticizers and adhesives widely used in products from children's toys and personal care products to building materials (eg, vinyl and plastic tubing), cosmetics, and pharmaceuticals. The ubiquitous use of phthalates in commercial products results in widespread general population exposure, which is of particular concern in susceptible subjects such as pregnant women, infants, and older children (45). In a study performed among newborns enrolled in a multiethnic birth cohort at the Mount Sinai School of Medicine in New York City, maternal urinary concentrations of phthalate metabolites and behavior were assessed within 5 days of birth. There were strong, inverse associations between increasing concentrations of high-molecular-weight phthalate metabolites and attention, orientation, and alertness among girls. Among boys, there was a slight positive association between increasing

low-molecular-weight (LMW) metabolites and motor performance (46). Two other studies from the same cohort examined the associations between phthalate exposure and ADHD and autistic behaviors. Engel et al (46) reported more ADHD-like behaviors among 4- to 7-year-old children whose mothers had higher urinary levels of LMW phthalate metabolites during pregnancy, and Miodovnik et al (47) found autistic-like behaviors among 7- to 9-year-old children born to women with higher urinary LMW phthalate concentrations (47). Finally, in the Study for Future Families cohort, Swan et al (48) reported that prenatal phthalate exposure was associated with decreased masculine behavior in preschool boys.

Extensive research in rodent models has shown that phthalates primarily act as antiandrogens and impair testosterone production in Leydig cells (49); however, it has also been proposed that phthalates disrupt the endocrine system by interfering with thyroid homeostasis through various mechanisms including alteration of transcriptional activity of the sodium/iodine symporter, inhibition of the binding of T_3 to purified thyroid receptors, and inhibition of T_3-induced cell proliferation (50). Other possible mechanisms through which phthalates may act include interference of intracellular calcium signaling, disruption of peroxisome proliferator–activated receptors activation, and alteration of lipid metabolism (51). Likewise, involvement of phthalates in neurodevelopmental disorders is supported by animal studies that reveal phthalate induction of hypothyroidism, which leads to decreased intellectual capacity and ASD (51, 52).

BPA is a high-production compound used to produce plastics and epoxy resins found in food and drink linings, toys, medical tubing, and dental fillings and sealants. Biochemical assays have shown that BPA binds weakly to the nuclear estrogen receptor and that it can bind to the membrane estrogen receptor with affinity equal to that of estradiol and can affect estrogen, androgen, and thyroid signaling at low dose levels. A steadily growing body of literature in animal and human studies draws associations between low-level BPA exposure and neurobehavioral effects.

In a study of mothers and their children participating in the Health Outcomes and Measures of the Environment (HOME) Study from Cincinnati, Ohio, Braun et al (53) reported that gestational BPA concentrations were positively associated with aggression and hyperactivity in children; this association was stronger in girls than in boys. Using the same cohort, Yolton et al (54) recently reported that BPA levels in maternal urine from the HOME study were associated with higher hyperactivity in 2-year-old girls but not boys. These results suggest that developmental exposures to BPA may have sexually dimorphic effects on behavior later in life. In contrast, in a mixed-race mother-child cohort study conducted in New York City, Perera et al (55) reported that child urinary BPA concentrations at 3 years of age were associated with higher emotional reactivity scores in children. Finally, based on a cohort of Korean children aged 8 to 11, Hong et al (56) reported a positive relationship between concurrent child BPA urinary concentrations and attention problems. To date, however, most human studies of BPA have used cross-sectional (point-in-time) designs that offer suggestive results but cannot address the temporality of exposure and disease. Larger prospective cohort studies are needed to confirm and validate findings from cross-sectional human studies as well as findings in laboratory animals.

Animal studies have shown changes in the dopaminergic system in the forebrain due to BPA exposure (57, 58). These findings are in line with the suggestion that BPA acts via estrogen receptors to alter dopamine signaling, leading to hyperactivity and attention deficits in humans (59). Perinatal low-dose BPA exposure has been shown to result in sex-specific anxiety, depression, and hyperactivity in several rodent models. Low-dose BPA also results in memory impairment in rodent studies. BPA treatment of pregnant female mice altered neocortical patterning in adult offspring and modified gene expression in the fetal forebrain (60). In addition, prenatal BPA exposure has been shown to interfere with numerous endocrine pathways in experimental studies, including nonclassic estrogen signaling pathways (at very low BPA concentrations) and to affect thyroid hormone, which plays a critical role in fetal and neonatal brain development (61). Multiple studies have drawn links between BPA exposure, alterations in gene expression, and neurodevelopmental outcomes in rodents (for a review, see Ref. 31). In one recent study, researchers assessed gene expression changes in rat amygdala to investigate the ontogeny of behavioral effects associated with early life BPA exposure. Their analysis revealed BPA-induced effects on a suite of genes known to be involved in sociosexual behavior. The study reported that the genes, which include estrogen receptor beta (*Esr2*) and two melanocortin receptors (*Mc3r* and *Mc4r*), were down-regulated (62). These specific genes play critical roles in the function of oxytocin/vasopressin signaling pathways, which are associated with human affective disorders such as anxiety and depression. Therefore, this study lays a foundation for understanding the behavioral outcomes associated with BPA exposure in rodents. Several concerns and limitations emerge from this review of exposures to nonpersistent plastic chemicals and their metabolites. The first is the small number of studies with similar health endpoints. Future researchers should use comparable health endpoints and measurement instru-

ments to facilitate systematic reviews. The second is the potential for misclassification of exposure due to the short biological half-life of phenols, phthalates, and other chemicals in humans. Urinary concentrations are believed to reflect recent exposure over the past 6 to 12 hours. Therefore, a single spot urine sample may not accurately classify long-term exposure (over weeks, months, or years) because data show that exposures are often episodic and vary over time (63). Additional large prospective cohort studies are needed to confirm and validate findings from cross-sectional human studies as well as findings in animals.

Heavy metals

Emerging evidence suggests that heavy metals, such as cadmium, arsenic, mercury, lead, and zinc, have ED properties as well as many other toxicological properties. For instance, studies have shown that metals can interfere with protein folding and protein-protein interactions, thus disrupting receptor-mediated endocrine activity (64). A substantial body of literature on the effects of metals on the endocrine system as well as on neurodevelopment exists. Numerous studies demonstrated the effects of low doses of metals on concentrations of gonadotropins, prolactin, ACTH, GH, and TSH. However, whether various effects of metals on neurodevelopment are related to ED or non-ED properties is still unclear and needs much more rigorous study (14, 65). In addition, metals may potentiate the effect of other EDCs or vice versa, even in cases where the actions of metals on neurodevelopment are not related to ED properties.

Preliminary work from the Mount Sinai School of Medicine on a longitudinal birth cohort in Mexico City demonstrates an association between heavy metal exposure and the adrenal stress hormone cortisol. Researchers found that heightened blood lead levels during pregnancy are associated with higher levels of maternal salivary cortisol when measured as a daily rhythm (66). These results are consistent with animal research suggesting that lead exposure disrupts cortisol metabolism. The research in pregnant women is important because cortisol has been shown to cross the placenta and could influence the health of offspring. These studies drive home the need for further investigation into the combined effects of social and physical environments to adequately identify the human health risks associated with various neurotoxicants (67, 68).

In general, heavy metals are known to affect the five steroid receptor pathways (estrogen, progesterone, testosterone, corticosteroids, and mineralocorticoids), as well as retinoic acid receptors, thyroid hormone receptors, and peroxisome proliferator–activated receptors (69). For example, cadmium has been shown to decrease calmodulin protein, which modulates calcium/calmodulin-dependent kinases, enzymes involved in the regulation of neurotransmitter synthesis, neurotransmitter release, and synaptic plasticity (70). Likewise, in rodents, arsenic and its metabolites have been found to reduce expression of GluN2 subunits (NR2A) (71) in *N*-methyl-D-aspartate receptors, which are involved in synaptic plasticity, learning, and memory (72). The abundance of heavy metals extant in the environment today due to mining, metal smelting, and other commercial properties maintains the need for further research into the mechanisms of these toxicants.

Pesticides

Pesticides are chemicals used to kill or deter insects, weeds, rodents, and other pests. Some pesticides are neurotoxins specifically designed to target enzymes that regulate neurotransmitters. It is not surprising that researchers are finding that these chemicals, which are designed to disrupt the biology of living organisms, have widespread effects on neurodevelopment in humans. In the CHAMACOS study, a longitudinal birth study of an agriculture-dependent community in the Salinas Valley, researchers are investigating the effects of pesticide exposure on neurodevelopment and overall child health. Marks et al (73) have shown that organophosphate pesticide exposure was significantly associated with ADHD for 5-year-old children. In the same cohort Eskenazi et al (74) found that with an exposure to two different types of DDTs, 12- and 24-month-old children had a 2- to 3-point decrease in Mental Developmental Index scores. Prenatal exposure to chlorpyrifos (CPF), a widely used broad-spectrum organophosphate pesticide, has been linked to a host of developmental and cognitive effects, including reduced head circumference and birth weight, abnormal reflexes in neonates, attention deficits in children, and neurodevelopmental abnormalities and significantly reduced childhood IQ by 5 to 7 points (73, 75–82). A recent study investigated links between CPF exposure and brain structure in children aged 5.9 to 11.2 years from a community-based cohort (83). Magnetic resonance imaging of the subjects revealed enlargement of multiple brain regions in individuals with high CPF exposure, specifically in regions that are critically involved in various cognitive and behavioral processes including attention, social cognition, and reward, emotion, and inhibitory control (84–87).

There are several proposed causal mechanisms for the observed alterations in neurodevelopmental disorders and abnormalities as a result of exposure to pesticides. These mechanisms include the inhibition of acetylcholinesterase and γ-aminobutyric acid, as well as decreased T_4, the inhibition of T_4 to T_3 conversion, and the prevention of iodine uptake. The studied effects of pesticides on neuro-

development illustrate the need for more consideration and observation of pesticides as EDCs (88).

Chemical mixtures

Very few data on how exposure to combinations of EDCs may affect neurodevelopment exist, yet in real-world scenarios in both environmental and occupational settings, people are typically exposed to combinations of chemicals. Interactions of EDCs may have additive, synergistic, or antagonistic effects. For example, PAH compounds are complex mixtures that are products of a variety of industrial processes. Engraff et al (89) observed that different combinations of PAH mixtures had toxicological effects that were markedly different from those of single PAHs (89). In addition, exposure to mixtures of chemicals during critical periods of development, such as the fetal period and early childhood, may have different and more severe effects than in later stages of development. For instance, in one study, researchers investigated whether coexposure to manganese and lead causes more severe impacts on neurodevelopment than exposure to the individual metals alone. The researchers analyzed blood samples from 455 children at birth and followed them until 36 months of age. The study reported that lead toxicity was higher in individuals exposed to high levels of manganese (90).

A few studies also indicate that the joint effects of environment and social setting play an important role in overall health. One study investigating neurodevelopment and cognition in the Columbia Center for Children's Environmental Health (CCCEH) cohort found that maternal exposure to environmental tobacco smoke during pregnancy led to reduced scores of mental development at age 2 and that the effect was greater in individuals from low-income and minority populations (91).

Research Challenges in Assessing Exposure and Health Effects of EDCs

Exposure assessment of EDCs represents one of the most significant challenges for moving this field forward. Epidemiological studies that examine the health impacts of EDC exposure are confronted by the difficulty of assessing exposure of short-lived chemicals during critical developmental periods such as pregnancy and infancy. However, some major categories of EDCs such as PCBs, DDT, and dioxins are classified as persistent organic pollutants due to their ability to persist, sometimes for decades, in humans, wildlife, and the environment. These long-lived EDCs are more amenable to studies linking exposures and detectable outcomes than the many EDCs that are rapidly metabolized in humans and wildlife and/or quickly degraded in the environment. Therefore, there is generally more confidence in findings from population studies analyzing the health effects of EDCs that have focused on the persistent pollutants. The tremendous variation in biological and ecological persistence of EDCs can lead to measurement errors or exposure misclassifications and cause inconsistencies among human studies and between animal models.

Another complicating factor for epidemiological studies of EDCs that relates to the standardization of exposure measurements across chemicals involves chemical half-life and method of sampling. EDCs and breakdown products of EDCs may be assessed in blood, hair, urine, milk, air, and even teeth. However, the concentration of a chemical in urine, measured in minutes, hours, or days, represents a distinctly different time frame than breakdown products analyzed in hair or teeth, which may represent exposures over periods of months or years. These variables make it difficult to compare exposures within and between studies. A better understanding of the variability of urinary biomarkers can help investigators classify exposures over a specific timeframe. The next generation of epidemiological studies would be enhanced by better exposure assessment strategies, including long-term (24-hour) collections and pooling multiple spot urine samples or identifying new matrices for measuring chemical exposure (92, 93). Because of the episodic nature of nonpersistent exposure to chemicals and their short half-lives, repeated single-spot urine samples exhibit substantial within-person variability. Studies may incorrectly identify biomarkers when they rely on a single urine sample to classify exposure over even relatively short periods of time, such as during pregnancy (94).

There are also many challenges related to assessment of neurodevelopmental outcomes. It may be necessary for researchers to incorporate interdisciplinary approaches to assess neurodevelopmental outcomes such as applying methods typically used in developmental psychology to study early life cognitive development. In addition, advances in imaging and biomonitoring technologies are allowing researchers to measure real-time prenatal brain development in association with environmental exposures.

Finally, it is critical that we better understand the scientific principles of how low-dose exposures of EDCs influence human health, particularly during vulnerable periods of development (Figure 2). Many studies suggest that, similar to hormones, EDCs are capable of eliciting nonmonotonic dose responses and that very low concentrations of EDCs can effect endpoints such as cell proliferation and organ development (95, 96). The duration, timing, and route of exposure may also have a big influence on how the chemical is metabolized and whether or

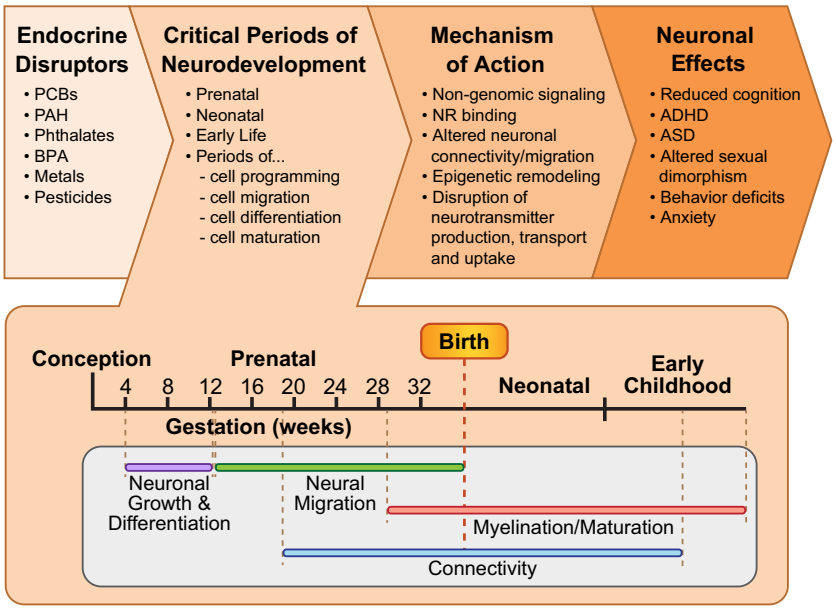

Figure 2. Schematic diagram illustrating how exposure to EDCs during critical periods of development can lead to neurodevelopmental disease.

not the chemical remains biologically active. This is most important during fetal development because of incomplete formation of the blood-brain barrier and during infancy, childhood, and adolescence, which are marked by continued maturation of key endocrine systems and are therefore extremely sensitive to perturbation by chemicals with hormone-like activity.

Future Directions

As research sheds light on the exquisite vulnerability of the fetal period and early childhood years to neurotoxicants, it is becoming increasingly clear that the timing of exposure to environmental toxicants may be even more important than the dose (97). Endocrine disruption that occurs at critical periods of development in utero or early childhood may alter development, whereas the same levels of exposure in fully developed brains may have little effect. One general mechanism by which prenatal and postnatal exposures could be linked to neurodevelopmental changes later in life is through the alteration of epigenetic markers, which have a central role in determining the functional output of the information that is stored in the genome (98). Although there are several epigenetic factors that can modify DNA to alter gene expression, such as histone remodeling and regulation by small noncoding RNAs, recent focus has been on the ability of environmental chemicals to reprogram DNA through changes in methylation patterns (23). In addition, synergistic effects from mixtures of EDCs or mixtures of EDCs and other pollutants may have critical impacts during these early life stages. It is of utmost importance to develop a more comprehensive picture of the effects of EDCs at different time points and dosages to limit or prevent exposures that could be detrimental to pregnant women and children.

Understanding responses to mixtures of chemicals that are representative of real-life scenarios is paramount. Epidemiological studies of human populations offer the opportunity to address questions of real-time exposure to chemical mixtures within a noncontrolled environment. However, these studies present many challenges, such as high degrees of correlation between at least some of these exposures, nonuniform data distributions, and a paucity of toxicological data for use in constructing models. New methods for epidemiological analysis of mixtures are being developed, but it is not known how well they perform or how they compare with conventional epidemiological approaches (90). Furthermore, although human studies often offer correlation between exposures and disease, animal studies are essential for developing data-driven mechanistic details, which are needed to provide a framework for targeted interventions, strategies for treatment, and changes in health policy. Furthermore, ecological studies in animals in their natural habitat are also useful and can serve as good parallels to human studies. And, although much research attention has been given to gene-environment interactions, it is becoming increasingly apparent in the literature that the social environment (including stress) can modify chemical toxicity and that such joint effects should be further explored (99, 100).

Researchers are now exploring the effects of environmental chemicals on neurodevelopment using new tools and technologies to more accurately measure exposure levels, to identify biological pathways, and to identify the most vulnerable populations. Interdisciplinary efforts between the neurobiology and environmental science communities aimed at addressing these questions could help shed light on how environmental toxicants may trigger underlying biological mechanisms. This insight will also help researchers better understand some of the current inconsistencies between animal models and human studies related to chemical effects on behavioral systems. In addition, synergistic efforts between these communities

could lead to more effective identification of risk factors and development of models to understand interactions between toxicants and their effects on genes during pregnancy and early childhood development.

In summary, the consequences of endocrine disruption during critical periods of neurodevelopment are far-reaching, with potential adverse impacts on social and behavioral functioning and cognitive development including impaired IQ and school performance. A converging body of research from animal models, clinical observations, and human population studies implicates EDCs in an array of neurodevelopmental disorders, behavioral problems, and adverse cognitive effects. Additional attention is needed to identify and understand the pathways through which EDCs affect neurodevelopment and to develop appropriate prevention and intervention strategies.

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