Executive Summary to EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals

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This Executive Summary to the Endocrine Society's second Scientific Statement on environmental endocrine-disrupting chemicals (EDCs) provides a synthesis of the key points of the complete statement. The full Scientific Statement represents a comprehensive review of the literature on seven topics for which there is strong mechanistic, experimental, animal, and epidemiological evidence for endocrine disruption, namely: obesity and diabetes, female reproduction, male reproduction, hormone-sensitive cancers in females, prostate cancer, thyroid, and neurodevelopment and neuroendocrine systems. EDCs such as bisphenol A, phthalates, pesticides, persistent organic pollutants such as polychlorinated biphenyls, polybrominated diethyl ethers, and dioxins were emphasized because these chemicals had the greatest depth and breadth of available information. The Statement also included thorough coverage of studies of developmental exposures to EDCs, especially in the fetus and infant, because these are critical life stages during which perturbations of hormones can increase the probability of a disease or dysfunction later in life. A conclusion of the Statement is that publications over the past 5 years have led to a much fuller understanding of the endocrine principles by which EDCs act, including nonmonotonic dose-responses, low-dose effects, and developmental vulnerability. These findings will prove useful to researchers, physicians, and other healthcare providers in translating the science of endocrine disruption to improved public health. (*Endocrine Reviews* 36: 593–602, 2015)

Introduction

In 2008, The Endocrine Society convened a group of experts to review the state of the science on endocrinological effects of environmental endocrine-disrupting chemicals (EDCs), leading to the landmark Scientific Statement on EDCs published in 2009, called hereafter "EDC-1" (1). EDCs are the hundreds or more "exogenous chemical(s), or mixtures of chemicals, that interfere with any aspect of hormone action" (2). In the subsequent 5 years, the field has moved forward substantially. Four lines of research have particularly influenced our knowledge about EDCs: 1) studies describing the consequences of EDC exposure on development and physiology (mainly conducted in rodent models, but with some notable exceptions in sheep and nonhuman primates); 2) reports investigating the mechanistic underpinnings of these disorders (gene expression and epigenetic changes induced in cell and tissue culture, together with molecular and cellular work conducted in endocrine tissues of EDC-exposed animals); 3) work documenting associations between body burdens of certain EDCs with disease propensity in humans (mainly epidemiological work); and 4) investigations of humans with known occupational or acute exposures to a particular chemical or group of chemicals with EDC activity. In 2015, there is far more conclusive evidence about whether, when, and how EDCs perturb en-

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Abbreviations: BPA, bisphenol A; DOHaD, developmental origins of health and disease; EDC, endocrine-disrupting chemical; ER, estrogen receptor; PBDE, polybrominated diethyl ether; PCB, polychlorinated biphenyl; PPAR, peroxisome proliferative-activated receptor.

docrine systems, including in humans. Thus, it is more necessary than ever to minimize further exposures, to identify new EDCs as they emerge, and to understand underlying mechanisms in order to develop methods to enable interventions in cases of EDC-associated disease. This is especially important because new chemicals may be released into the marketplace without appropriate safety testing.

The second Scientific Statement on EDCs, "EDC-2" (3), focuses on a subset of endocrine systems for which there have been the greatest research advances over the past 5 years: obesity, diabetes mellitus, and cardiovascular diseases; female and male reproduction; certain hormone-sensitive cancers; the prostate gland; the thyroid; and the brain, especially neuroendocrine systems and neurodevelopment. Among the known EDCs, several classes are particularly well-investigated, including bisphenol A (BPA), phthalates, herbicides, pesticides such as atrazine and DDT, and industrial chemicals such as polychlorinated biphenyls (PCBs) and polybrominated diethyl ethers (PBDEs). These and other EDCs have broad effects on multiple endocrine endpoints and act through a variety of mechanisms in the body. Furthermore, EDCs often have multiple congeners and metabolites that can affect the body through various mechanisms.

In this Executive Summary, we provide a synthesis of the key points discussed in detail in the main text of the parent article. Please note that references are given only minimally here; readers are referred to the parent article for all details and a comprehensive bibliography.

Characteristics of EDCs and the Importance of Developmental Exposures

A thorough understanding of basic endocrine principles about hormone actions is necessary to comprehend how EDCs perturb these physiological systems. Like hormones, EDCs exhibit complex dose-response curves, and they can act at extremely low concentrations. Of utmost importance is that hormone levels, receptors, and physiological responses change dramatically across the life cycle. During early development—the fetus and infant—exposures to proper levels of hormones are needed for normal ontogenic processes to occur. Too much or too little of a hormone can result in neurological impairments (eg, thyroid hormone deficiency), disorders of sexual development (eg, abnormal androgen or estrogen levels), or even death. Exposure to exogenous compounds such as EDCs that can mimic or antagonize these and other hormonal systems change the developmental trajectory. Depending on when an individual is exposed, the nature of the EDC, and its levels, an adverse outcome may be evident at birth, or there may be a latent disease outcome. This latter concept, often referred to as the developmental origins of health and disease (DOHaD), underlies latent effects of EDCs on every endocrine system studied to date. Increased knowledge of the role of EDCs in the DOHaD across multiple systems is due, in part, to a measurable shift in the DOHaD funding portfolio of the National Institute of Environmental Health Sciences over the past decade (4), as well as to changes in other funding agencies in the U.S. and internationally.

The mechanisms by which EDCs act in the body are quite complex because EDCs, unlike endogenous hormones, are not natural ligands and do not interact with hormone receptors with the same specificity and affinity. Nevertheless, EDCs interfere with those endogenous systems. Most prior research on EDCs focused on their actions on estrogen receptors (ER) and, to a lesser extent, antiandrogenic and thyroid effects. In the past 5 years, there has been increasing evidence for EDC effects on these receptor classes, along with other members of the nuclear receptor superfamily such as glucocorticoid and the peroxisome proliferative-activated receptor (PPAR) family. Beyond the nuclear receptor superfamily, more is now known about the effects of EDCs on nuclear and membrane receptors, including steroid membrane-associated receptors, peptide and protein receptors, and neurotransmitter receptors.

Part of the complexity of the EDC field is that exogenous chemicals are being added on top of the endogenous hormonal milieu, such that complex mixtures, dose additivity, and synergism between and among hormones and chemicals are the norm. For example, Kortenkamp and colleagues (5) showed clearly that multiple xenoestrogens could modulate the effect of 17β-estradiol in a yeast estrogen system assay, although each of the xenoestrogens was present in a concentration below that in which they produced an observed effect. In addition, Nagel et al (6) showed how the relative binding affinity of a xenoestrogen for the estrogen receptor can be altered by the presence of serum binding proteins in the buffer. This work has two important implications. It demonstrates first that even "weak" estrogens can significantly alter estrogen action, and second, that there is no obvious threshold of effect. Thus, we should not think of chemicals acting singly, but rather in mixtures. The ER is likely to be an important target of mixture effects because there are many kinds of chemical structures that interact with the ligand binding domain of the ER (7), and estrogen signaling has important observable effects on reproduction. It is unclear to what extent the addition of a single protein is generalizable, but it should not be disregarded. Nevertheless, this kind of summation is not the only way that environmental

chemicals can interact with endogenous hormone systems to produce adverse effects.

EDCs also act on enzymes involved in steroidogenesis, steroid metabolism, and protein/peptide synthesis. They affect intracellular signaling processes and cell proliferation, growth, and death. There are hundreds, if not thousands, of papers showing that EDC exposures affect expression of genes and proteins in different cells, tissues, and organs. Recent evidence suggests that some EDCs may cause molecular epigenetic changes, including in the germline, which in turn may lead to transgenerational effects of EDCs on numerous organ systems. This latter point is important because it suggests that the legacy of EDC exposures may go beyond the individual and can last even if there is no further exposure or despite efforts to clean the environment.

From Cells to Animals to Human Health

Converging lines of evidence strongly support the contention that EDCs are contributing to diminished quality of life, increased cancer susceptibility, and endocrine diseases in humans. However, as is the case for any system in which the timing of exposures (eg, fetus) may occur years or even decades before the development of a disease, or when there are lifelong low-level exposures, the issue of causality is difficult to prove, especially in long-lived species such as humans. Nevertheless, there is increasing evidence that EDC exposures play a substantive role in disease causation or progression, or may alter the susceptibility to disease over a lifetime. Occupational or toxic exposures to single chemicals, eg, the Seveso, Italy, dioxin exposures; Agent Orange exposure in South Vietnam; and perfluorooctanoic acid contamination of the Little Hocking River, among others, are associated with documented health effects, including endocrine-sensitive disease endpoints. In addition, experimental animal research, primarily on rodents but with increasing frequency in primates, shows that low-level exposures to EDCs, especially during early development, lead to both transient and permanent changes to endocrine systems. This results in impaired reproduction, thyroid function, and metabolism, and increased incidence and progression of hormone-sensitive cancers. Considering that the endocrine systems of all mammals—including humans—are highly conserved, this biomedical research literature is highly translatable. Finally, a growing body of epidemiological studies is revealing associations between EDC body burdens and a variety of diseases. Much of this recent research has been conducted on mother-child cohorts and includes measures of prenatal or early postnatal exposure, something that is extremely important considering the DOHaD hypothesis.

Review Criteria for EDC-2

The authors of the Scientific Statement established several criteria in reviewing the literature and determining what articles to include or exclude. Searches focused on the literature of the past 5 years for those EDCs for which the evidence spans several levels of analysis, especially epidemiological information in humans, together with work on experimental animal data, particularly during development, and mechanistic and molecular information from cells and animal tissues after exposures. However, whereas nonmammalian models are an extraordinarily important part of the EDC literature, we focused entirely on mammalian work due to space constraints. Several types of EDCs, including BPA, pesticides, phthalates, and persistent organic pollutants such as PCBs and dioxins, were included in all of the sections. We also attempted to identify the critical research gaps regarding the effects of EDCs on human health. No article was selected for either inclusion or exclusion based solely on positive or negative findings; however, we did exclude studies that did not utilize adequate positive or negative control groups or populations or experimental work that was conducted at unrealistically high dosages. In a few cases, if such studies were discussed for illustrative purposes, we pointed out these caveats. Sample sizes needed to be high enough to have adequate power to draw accurate statistical conclusions using appropriate statistical methods. Some methodological concerns were also grounds for exclusion.

In summary, for this current review, it was ultimately the rigor of the science that led to an article's inclusion in EDC-2. To follow is a synopsis of the highlights from the seven areas of endocrine research that were reviewed in depth in EDC-2. For this Executive Summary, we have not provided a comprehensive list of references, but a complete bibliography of over 1300 articles reviewed in this Statement is provided at the end of EDC-2. Please note that some text from the full Statement is cited verbatim here, including the bullet-point summaries.

Obesity, Diabetes Mellitus, and Cardiovascular Diseases

- Disruption of glucose and lipid homeostasis is a risk factor for metabolic disorders including obesity and diabetes mellitus.
- BPA, phthalates, tributyltin, arsenic, PBDEs, perfluorooctanoic acid, dioxin, PCBs, and DDTs are known to have effects on cellular and animal models.
- In animal models, prenatal and perinatal exposures to some EDCs disrupt the homeostatic con-

trol of adipogenesis and/or energy balance, and induce obesity. A growing number of EDCs alter insulin production, secretion, and/or function, increasing the susceptibility for type 2 diabetes mellitus (T2D). Some animal models suggest that EDCs have direct adverse effects on the cardiovascular system.

- A number of cross-sectional epidemiological studies associate EDC levels with obesity, diabetes mellitus, and cardiovascular diseases in humans. There are important prospective studies associating exposure to persistent organic pollutants and T2D.
- Obesogenic and diabetogenic effects are induced in a nonmonotonic dose-dependent manner. Exposures to different levels produce diverse phenotypes.
- The molecular mechanisms involved are still largely unknown, but alteration of gene expression after binding to the aryl hydrocarbon receptor, PPARγ, and ERs seems to play a role.
- The interaction between EDC exposure and single nucleotide polymorphisms associated with obesity, T2D, and cardiovascular diseases is a key issue for future studies.

Research on the effects of EDCs on obesity, diabetes mellitus, and associated metabolic disorders is a relatively new discipline and was selected as a focus of EDC-2 because of the importance of this emerging area. T2D and obesity have interlinked and overlapping pathologies, but there is sufficient evidence to conclude that some EDCs act as obesogens and others act as diabetogens. There are relatively few studies on EDCs acting as cardiovascular disruptors, but we briefly reviewed this literature because we view this as an important emerging area. For the latter, recent evidence in animals suggests that BPA may directly target cardiomyocytes, trigger arrhythmias, and cause hypertension.

Both cellular and animal models demonstrate a role for EDCs in the etiology of obesity and T2D. For obesity, animal studies show that EDC-induced weight gain depends on the timing of exposure and the age of the animals. Exposures during the perinatal period trigger obesity later in life. New results covering a whole range of EDC doses have underscored the importance of nonmonotonic dose-response relationships; some doses induced weight increase, whereas others did not. Furthermore, EDCs elicit obesity by acting directly on white adipose tissue, although brain, liver, and even the endocrine pancreas may be direct targets as well.

Regarding T2D, animal studies indicate that some EDCs directly target β- and α-cells in the pancreas, adipocytes, and liver cells and provoke insulin resistance together with hyperinsulinemia. These changes can also be associated with altered levels of adiponectin and leptin—often in the absence of weight gain. This diabetogenic action is also a risk factor for cardiovascular diseases, and hyperinsulinemia can drive diet-induced obesity. Epidemiological studies in humans also point to an association between EDC exposures and obesity and/or T2D; however, because many epidemiological studies are cross-sectional, with diet as an important confounding factor in humans, it is not yet possible to infer causality.

EDCs and Female Reproduction

- The female reproductive system is complex and requires proper structure and function of many organs, including the ovary, uterus, vagina, and anterior pituitary. EDCs have the potential to interfere with female reproduction by adversely affecting the structure and/or function of female reproductive organs.
- Studies consistently show that some EDCs impair key processes in ovarian development (germ cell nest breakdown and follicle formation) in animal models, adversely affect the structure and function of the postnatal ovary by inhibiting follicle growth and/or increasing atresia/apoptosis in animal models, and disrupt steroid hormone levels in animals and women.
- Although the data are not always consistent between experimental and epidemiological studies, they suggest that some EDCs may adversely affect the structure and/or function of the uterus, vagina, and anterior pituitary. Some EDCs are also associated with abnormal puberty, irregular cyclicity, reduced fertility, infertility, polycystic ovarian syndrome, endometriosis, fibroids, preterm birth, and adverse birth outcomes.
- Limited information is available to explain the inconsistencies in findings among studies and to understand the mechanisms by which EDCs adversely affect female reproduction. Thus, more research is needed in this area.
- Many potential EDCs have not been studied at all in experimental or epidemiological studies. Thus, there is a real need for future studies to focus on the effects and mechanisms by which EDCs affect female reproductive outcomes in both experimental and epidemiological studies.

The literature over the past 5 years has added to an already robust body of evidence for links between EDCs and female reproductive impairments. All of the reproductive organs, including the brain, pituitary, reproductive tract, and ovary, are vulnerable to a variety of EDCs. This is not surprising, considering the high hormone sensitivity of these organs. Moreover, reproductive developmental periods, especially prenatal and early postnatal life and puberty, are vulnerable periods for high sensitivity to EDC exposures. Some areas of research that have moved forward in recent years have provided evidence for disruption of ovarian development, folliculogenesis and steroidogenesis, impairments of ovulation, and diminished quality of oocytes. Like the ovary, the uterus and vagina are highly sensitive to EDCs, with both structural and functional impairments caused by EDC exposures.

Reproductive functional processes including the timing of puberty, reproductive cycles, fertility and birth outcomes, and premature reproductive senescence, are associated with both prenatal and adult EDC exposures in animal and epidemiological studies. However, the literature linking specific EDCs to reproductive outcomes is somewhat inconsistent, possibly due to differences in windows of exposure, dosages, and which EDCs are detected in humans or used in animal models. Thus, more research is needed in this important area.

Finally, several reproductive pathologies have associations with EDCs. In particular, polycystic ovarian syndrome in humans is associated with higher body burdens of certain chemicals, especially BPA, and animal models corroborate this finding. Other disorders such as endometriosis, fibroids, and adverse birth outcomes have associations with higher EDC exposures in humans and animals.

EDCs and Male Reproduction

- Development of the male reproductive system is hormonally regulated, with androgens providing the driving force in masculinization of genitalia.
- Cryptorchidism and hypospadias are the most common birth defects of male reproductive organs, and their incidence has increased in many countries over the same period when the incidence of testicular cancer has multiplied.
- Although results are variable, a large proportion of men have decreased semen quality, resulting in delayed time to conception.
- Animal experiments demonstrate clearly that disruption of hormones regulating development of the reproductive tract causes cryptorchidism, hypospadias, poor semen quality, and ultrastructural testicular abnormalities that are similar to changes associated with human testicular cancer. These outcomes can therefore be considered signs of a common problem, testicular dysgenesis syndrome.
- Antiandrogens, xenoestrogens, and dioxins are the best-characterized endocrine disruptors of the male reproductive system. Antiandrogenic chemicals act additively, irrespective of their mechanism of action (ie, whether they are receptor antagonists or inhibitors of hormone synthesis).
- The number of known antiandrogenic and xenoestrogenic EDCs is increasing, and it remains to be established which of them might contribute to human reproductive problems.

The EDC field has some of its deepest roots in male reproductive biology. There is increasing mechanistic evidence that EDCs, especially those that are antiandrogenic, play key roles in impairments in the development and maintenance of male health. Some of the potential defects and diseases that have been associated with developmental exposure to EDCs are: hypospadias, cryptorchidism, testicular germ cell cancer, and poor semen quality. In combination, these defects are referred to as testicular dysgenesis syndrome, and it has been proposed that the increase in testicular dysgenesis syndrome is due in part to increased exposure to EDCs, especially during development.

Hormone-Sensitive Cancers in Females

- Incidences of breast (especially in men and women under 40 y of age), endometrial, and ovarian cancers are increasing, and it is suspected that EDCs and other environmental factors are contributing to this increase.
- Critical periods of mammary gland development enhance susceptibility to lifelong or persistent adverse effects of EDC exposure.
- Rodent studies have identified EDCs altering mammary development, susceptibility to tumors, and lactation after critical period exposures. Examples include industrial chemicals, pollutants, herbicides, and pharmaceuticals.
- Dioxin is an EDC demonstrating DOHaD principles; early life exposure leads to delayed pubertal breast tissue development in female rodents and in girls. Dioxin also has demonstrated effects on later life lactation and breast and ovarian cancer risk.

- Recent epidemiological studies focused on adult breast cancer risk indicate the importance of evaluating EDC exposures at young ages when assessing hormone-sensitive cancers in women.
- Mechanisms of action for female reproductive cancers are not well understood; the roadblocks result from the innate complexity of tissue-specific multicell communication, lack of appropriate in vitro test systems, variables such as obesity and cyclicity, and latency (often 2–3 y in rodents and 40–50 y in women) from the time of exposures to adverse outcomes.
- Because many targets of EDCs are conserved across species, future epidemiological studies focusing on mechanisms of action for cancer of the breast, ovary, or uterus should test reported rodent biomarkers of effect and vice versa to expand translation of effects.
- There is a critical need for testing mixtures of EDCs based on their structural or activity homology and for integrating adequate precancerous cell lines representing female reproductive tissues in high-throughput chemical screening and testing.

The incidence of hormone-sensitive cancers of the breast, uterus, and ovary is on the rise, and it has been hypothesized that increases in these types of cancers are, in part, due to EDCs in the environment. Research on the effects of EDCs on breast development and associations with mammary cancer has increased over the past few years, with both epidemiological and rodent studies supporting such links. The mammary gland is highly responsive to hormones, and its development can be permanently changed through abnormal hormonal environments. Among the EDCs with the strongest associations to impaired mammary development are dioxins, pesticides such as DDT and DDE, BPA, phthalates, and perfluorooctanoic acid. Other cancers such as uterine and ovarian cancers also have reported links to EDC exposure and merit further research because the studies published to date have identified EDCs with estrogenic/antiestrogenic actions.

EDCs and the Prostate Gland

The following key points summarize the links between EDCs and prostate health:

- The prostate gland is a hormone-dependent structure, and dysregulation of hormonal signaling is a known contributor to the high rates of prostate disease with aging. A number of EDCs have been associated with aberrant prostate growth, making it a likely EDC target.
- Disruption of multiple hormonal pathways by EDCs has been identified in the prostate, including ERs, androgen receptor, vitamin D receptor, retinoic acid receptor/retinoid X receptor, prolactin, and steroid-metabolizing enzymes. This may underlie increased prostate cancer risk related to certain EDCs.
- Gene-environment interactions have been identified for several pesticides in population studies and suggest that certain genetic alterations such as single nucleotide polymorphism variants may predispose subpopulations of men to heightened prostate cancer susceptibility from exposure.
- EDC classes with known prostatic effects include pesticides, insecticides, herbicides, Agent Orange chemicals, PCBs, alkylphenols, BPA, and some heavy metals.
- Epidemiological evidence indicates increased prostate cancer rates and mortality in men exposed to specific pesticides, Agent Orange, alkylphenols, PCBs, and inorganic arsenic.
- Animal models and human cell-based studies provide evidence for elevated prostate cancer risk from BPA exposure, with increased sensitivity to BPA reprogramming during early-life development.
- Cell-based and animal studies support the human data, extend to additional EDCs of concern, and identify cellular (eg, stem cells) and molecular (eg, epigenetics) pathways that underpin increased prostate disease risk.
- Occupational pesticide/herbicide exposure levels provide greater prostate cancer risk, although emerging evidence is suggestive of general population risk in areas of pesticide/herbicide utilization. PCB risk appears limited to occupational exposure levels, and arsenic risk occurs in hotspot areas of high inorganic arsenic in drinking water. BPA effects in animal models have been noted at low doses equivalent to general population exposures.
- Future studies are needed to identify prostate disease risk with low-dose EDC exposures, identify critical life periods of exposure vulnerability, investigate adverse effects of several unstudied EDC classes that interfere with steroid actions, and elucidate modes of action for EDC prostatic effects and discovery of biomarkers of EDC exposure to identify at-risk populations.

Since the publication of the EDC-1, data continue to emerge that identify the prostate gland as a likely direct EDC target tissue. As is the case for other hormone-sensitive systems, exposures of the prostate gland, especially during development, result in lifelong changes that influence disease susceptibility and contribute to prostate cancer incidence, progression, and mortality. Undoubtedly, the high sensitivity of the prostate to EDC exposures is due to the absolute dependence of this gland on androgens and multiple other hormones that regulate its development, secretory activity, and homeostasis throughout life, with hormone imbalances contributing to disease ontogeny in this structure.

Accumulating data confirm that the adult human prostate is directly affected by EDCs, with increased cancer rates and mortality occurring in men exposed to pesticides, Agent Orange chemicals, alkylphenols, and trace metals, all with known hormonal actions as a common factor. New findings in human and animal models also support previous data showing a developmental window of heightened sensitivity to EDCs resulting in increased susceptibility to aging-associated pathologies including carcinogenesis. Modes of action include altered steroid receptor levels and actions, changes in steroidogenic enzyme activity, and epigenomic reprogramming that may underpin an increased disease propensity throughout life. Additionally, important new data identify the stem and progenitor cells within the developing and adult prostate gland as direct targets of EDC actions, further contributing to long-term memory of prior exposures within the gland. Because these cells populate the prostate during embryonic development, maintain adult homeostasis, and repopulate the prostate after acute or chronic injury, and because stem-like cancer cells play a fundamental role in tumor progression, reprogramming of prostate stem and progenitor cells by EDCs has marked potential for transmitting lifelong changes within the gland, including predisposition to diseases with aging.

Thyroid Disruption

- A large number of chemicals and chemical classes are known to affect the thyroid system.
- Animal studies have also demonstrated that a number of chemicals, including but not limited to PCBs, PBDEs, some phthalates, and perchlorate, can reduce circulating levels of thyroid hormone. Interestingly, not all of these chemicals also cause an increase in serum TSH.
- Some chemicals that affect the thyroid system in animals have been shown to be associated with cognitive deficits in humans. However, exposure is not always correlated with reductions in thyroid hormone in humans.
- Thyroid hormone produces different effects at different developmental stages—in humans as well as in animals—and the consequences of disruption are stage-specific.
- Some chemicals clearly exert actions on the thyroid system in humans and animals at environmentally relevant concentrations. The mechanism(s) by which chemicals can produce this effect varies.
- Three key areas of research are urgently needed: 1) identify biomarkers of thyroid hormone action in tissues to test the ability of chemicals to interfere with hormone action in the absence of effects on serum hormone concentrations; 2) determine whether chemicals with different mechanisms of action on the thyroid system can synergize to cause adverse effects; and 3) identify high throughput assays that predict thyroid "disruption."

Thyroid hormone is essential for normal development and for the control of many aspects of adult physiology in vertebrates. There is a growing list of synthetic chemicals that can interfere with thyroid function or thyroid hormone action. There are several important conclusions to be drawn from the recent data on thyroid disruption. First, there is good evidence from animal, biochemical, and human studies that specific chemicals can interfere with thyroid hormone action and cause adverse effects at the population level. Second, the current biomarker of thyroid "disruption"—circulating levels of thyroid hormone—may not be faithfully reflecting EDC effects on thyroid hormone action in tissues.

Specific EDCs have been shown to interfere with a number of the pathways involved in thyroid hormone biosynthesis, metabolism, receptor activation, and function. Recent publications have demonstrated effects of EDCs on iodide uptake and organification, thyroid hormone interactions with its distributor proteins in the blood, and metabolism and cellular transport of thyroid hormone. Developmental exposure is particularly pertinent to the thyroid axis because neural development is impaired by thyroid perturbations. Evidence from rodents supports cognitive and other behavioral impairments caused by prenatal EDC exposures that interfere with thyroid hormone action. Epidemiological data in humans support cognitive deficits in children and diminished IQ in children exposed to certain thyroid disruptors prenatally.

Neurodevelopmental and Neuroendocrine Effects

- The experimental animal literature consistently shows that the structure and function of the brain's neuroendocrine systems can be altered by developmental exposures to EDCs.
- The adult neurobiological consequences of developmental exposures include alterations in peripheral hormones and changes in behaviors.
- Underlying mechanisms of EDC actions in the brain include molecular and cellular changes in expression of particular genes and proteins involved in neuroendocrine and other behaviors, including those involved in cognitive and affective functions.
- The brain is highly vulnerable to EDC exposure because of the widespread distribution of nuclear hormone receptors, steroidogenic enzymes, and neurotransmitter systems on which EDCs can act.
- Strong experimental evidence in animals shows that there are sex differences in EDC effects on the brain. Moreover, epidemiological work also shows that relationships between body burdens of chemicals and particular behaviors often differ between the sexes in human studies.
- In humans, epidemiological data support associations between higher exposures to EDCs and decreased IQ, increased neurodevelopmental problems, and other neurocognitive outcomes.
- Future research needs to focus on sex differences in endocrine disruption of the brain and to consider both age of exposure and age at assessment in interpreting results.

The brain is exquisitely sensitive to a wide range of hormones, making it yet another important target of EDCs. Its widespread and heterogeneous expression of nuclear and membrane hormone receptors and steroidogenic enzymes begins early in development and undergoes dynamic change throughout life. Neural and glial cell birth, maintenance, and death—processes involved in neural and synaptic plasticity—are hormone sensitive. The brain's neurotransmitter systems are also modulated by steroid hormones. In the past 5 years, these areas of research have expanded in experimental animal models, often relating exposures to molecular or structural change, together with a functional outcome such as neurobehavior. Recent papers additionally include evidence for molecular epigenetic effects of EDCs on neurodevelopment. Previously in EDC-1, we discussed some specific actions of EDCs on the neuroendocrine systems of the hypothalamus controlling reproduction; that literature has expanded to other neuroendocrine control systems that regulate the body's peripheral endocrine hormones, which in turn are modulated by hormone feedback in the nervous system.

Much recent work on EDC actions on the brain has utilized the DOHaD model of developmental exposures on outcomes assessed later in life as related to brain and behavior. A salient feature of more recent work is the use of lower, more ecologically and biomedically relevant dosages of EDCs and their relationship to the behavioral phenotype and underlying structural and function changes to the nervous system. Important epidemiological studies on the neurobehavioral and neurodevelopmental effects of EDCs have also been published since the EDC-1, linking EDC body burden to the likelihood of having neurobehavioral problems or longitudinal studies conducted on various populations around the world.

Conclusion and Recommendations

As stated in the full EDC-2 Scientific Statement, the past 5 years represent a leap forward in our understanding of EDC actions on endocrine health and disease. The scientific literature published during this period has provided much deeper insights into the underlying molecular and cellular mechanisms of action, the importance of critical developmental exposure periods, and stronger epidemiological studies in humans from around the world. Despite the advances that have been made since EDC-1, more mechanistic and dose-related information is needed. In addition, research needs to be more strongly translated from animal models to humans. Although it is widely accepted that the hormone signaling pathways of mammalian systems are highly conserved, investigators need to take novel approaches to more directly infer cause and effect in humans. In addition, due to obvious limitations in human work, there are still gaps in knowledge in the basic biology of hormones and their actions during fetal development. This merits inclusion of work in nonhuman primates and capitalizing on other animal models such as transgenic mice, sheep, and other large animals. The concept of sensitive life stages should be invoked in all experimentation and investigation, whether human or animal, keeping in mind that not only the timing of exposure, but also the sex and life stage at evaluation, will influence results. The body's endocrine and neural systems are under constant dynamic change throughout the life cycle, with an effect observed at one stage not necessarily paralleling the effect at another. As new chemicals are disposed of, used as pesticides, in food and water storage products, and in other applications with which humans come in ubiquitous contact, we must conduct research to

determine whether or not these chemicals have EDC actions in order to avoid adding to the exposure burden.

Recommendations for research over the next 5 years

- Extend mechanistic studies of EDC actions on nuclear hormone receptors beyond ERs, androgen receptor, progesterone receptor, glucocorticoid receptor, and thyroid hormone receptors, and PPARs to other nuclear hormone superfamily members and to membrane steroid hormone receptors.
- Investigate EDC effects on enzymes involved in steroidogenesis, hormone metabolism, and protein processing in humans and animal models.
- Consider tissue-specific effects of EDCs.
- Translate research from rodents into nonhuman primates, sheep, and other species; take advantage of transgenic (especially humanized) animals, keeping in mind the need for a better understanding of hormones and early life development in humans.
- Test additional critical periods beyond prenatal and early postnatal— eg, adolescence as an additional sensitive developmental window.
- Evaluate EDC outcomes at different life stages— not just adulthood.
- Design studies to consider sex and gender differences in response to EDCs.
- Perform longitudinal and multigenerational analyses in animals and humans.
- Evaluate and implement emerging and sensitive testing systems, including high-throughput systems, for hazard assessment, screening, and prioritization.
- In humans, consider genetic diversity and population differences in exposures and outcomes. This should include racial, ethnic, socioeconomic, and geographic variables.
- Expand research to emerging "EDCs of interest" and to mixtures of low-dose EDCs.
- The team science approach, including teams of basic, translational, and clinical scientists, epidemiologists, health care providers, and public health professionals, needs to be a priority for future research and funding.

Recommendations beyond research for the next 5 years

Ten years ago, there simply was not the body of evidence that there is today about the endocrine actions and disease consequences of EDCs. The previous review of the literature in EDC-1 was a first step in providing such evidence, and the expansion of data reviewed in EDC-2 removes any doubt that EDCs are contributing to increased chronic disease burdens related to obesity, diabetes mellitus, reproduction, thyroid, cancers, and neuroendocrine and neurodevelopmental functions. Several next steps are needed. Scientific societies have already played important roles in communicating the risks to physicians and patients and providing materials to help people minimize exposures. This needs to continue, with primary care physicians, endocrinologists, pediatricians, and reproductive medicine practitioners being provided educational materials for their practices. Responsible journalists have played a surprisingly important role in communicating to the general public that EDCs are a real concern, and the public has responded by demanding the removal of some of these chemicals from consumer products. However, replacement products can be introduced without disclosing what those products are or whether they have been appropriately tested, calling into question the effectiveness of regulation of potential emerging EDCs. Dialogs need to continue with governmental agencies involved in monitoring, assessing, and regulating EDCs around the world. Chemicals should be safety tested before sale on the open market, and this should include testing for endocrine-like activities at low doses. Although certain countries and a subset of states in the United States have imposed some bans, the fact that EDCs are ubiquitous makes it a global issue that requires international partnerships among developed and developing nations.

As we conclude EDC-2, we turn to the topic of whether and how new chemicals should be dealt with. In EDC-1, we invoked the precautionary principle; here, we briefly discuss the terminology and philosophy utilized by some organizations. The US National Toxicology Program (NTP) uses the phrase "evidence integration" (http://ntp.niehs.nih.gov/pubhealth/hat/noms/index-2.html) in determining whether a chemical may have an impact on human health. The NTP has recommended this terminology to the National Research Council and the US Environmental Protection Agency (http://www.nap.edu/catalog/18764/review-of-epas-integrated-risk-information-system-iris-process). When high-quality endocrinological studies demonstrate that a chemical interferes with hormone action in vivo and in vitro at environmentally (human) relevant concentrations, and when we have a high degree of evidence that these hormone systems are essential for normal development, it is reasonable to infer that these chemicals will produce adverse effects in humans. This inference is scientifically based, but it is often considered to be "precautionary" because overt adverse effects may not have been fully characterized. Where to set the bar for evidence of hazard and

risk represents a balance of "precautionary principles" designed to protect industry and protect public health.

There are other points that several of the authors of this statement would like to raise with respect to precaution. It simply is not reasonable to assume a chemical is safe until proven otherwise. Clearly, not all chemicals are EDCs, but substantial information needs to be provided before inclusion of a new compound in a food storage product, a water bottle, health and beauty products, or a household product. Replacement chemicals provide excellent examples of why precaution is merited. The BPA substitute, BPS, is now shown to have endocrine-disrupting activity on par with BPA in experimental studies discussed in EDC-2. A further need for precaution is based on evidence that individuals exposed to EDCs may carry that body burden for their entire lives in the case of long-lived chemicals; that even short-lived chemicals may induce changes that are permanent; and that some actions of EDCs are observed in an individual's offspring. Transgenerational effects of EDCs mean that even if a chemical is removed from use, its imprints on the exposed individual's DNA may persist for generations and possibly forever. These observations, which have cut across all areas of EDC research reviewed in EDC-2, make it paramount to evaluate any new chemical before inclusion on the open market in any form in order to avoid any further contribution to the problem.

Recommendations beyond research for the next 5 years include:

- Educate the public, the media, politicians, and governmental agencies on ways to keep EDCs out of food, water, and air and to protect developing children in particular.
- Develop industrial partners such as "green chemists" and others who can create products that test and eliminate potential EDCs.
- Recognize that EDCs are an international problem and develop international collaborations.
- Cultivate the next generation of EDC researchers, green chemists, physicians, and public health experts with expertise in endocrine systems.
- Funding agencies need to go beyond the "one scientist, one project" and "one clinician, one patient" perspective to fund team science and healthcare.
- Funding agencies need to prioritize EDC research in the basic, clinical, and epidemiological realms, especially considering that the cost of research and prevention will result in substantial cost savings in treatment and mitigation.
- Emphasize the need for precaution and prevention.
- Determine how much evidence is enough based on rigorous, peer-reviewed science— keeping in mind that absolute proof of harm or proof of safety is not possible.

Acknowledgments

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