TURNING POINT

Endocrine Disruptors

From theoretical and experimental biology to a public and environmental health policy

Ana M. SOTO*, Cheryl SCHAEBERLE, Carlos SONNENSCHEIN*
Dept of Immunology, Tufts University School of Medicine, Boston MA 02111, * Centre Cavaillès, École Normale Supérieure, Paris 75005

Abstract

Over three decades ago, the term "endocrine disruption" was conceptualized at the Wingspread Conference (1991). The work of our team (Tufts University School of Medicine, Boston, MA, USA) on the presence of estrogen in plastic compounds provided crucial findings. A scientific interdisciplinary field emerged from the proposed foundational concepts. The idea that development was a " program " was being contested by the emergent field of ecological developmental biology at that time, thus creating a situation of theoretical dissonance. Despite this context, our research field produced new concepts and a body of evidence documenting the deleterious health effects of endocrine disruptors. A number of regulatory measures have been enacted in Europe over the last decade. The slowness of this action is a consequence of regulatory inertia, lobbies against regulation efforts, and theoretical ambiguities that plague researchers and are cleverly exploited by pressure groups. In 2012 the Endocrine Society proposed that principles of endocrinology should guide research and regulation. To further this reasonable proposal, we propose to dig deeper into the theoretical bases of biology to identify sound and precise theoretical principles. Indeed, according to Ludwig Boltzmann, "nothing is more practical than a good theory". This applies not only to conducting research, but also to effectively blocking the pernicious effects of those who seek to hinder the creation of a protective health policy by creating doubt and ignorance.
Keywords: xenoestrogens, bisphenol-A, fetal origins of adult disease, transgenerational inheritance, biological theory, organicism, ecological developmental biology (eco-devo)

Résumé

Perturbateurs endocriniens : de la théorie et de la biologie expérimentale à un politique de la santé publique environnementale

Il y a une trentaine d'années, les experts et scientifiques réunis à la conférence de Wingspread (1991) contribuèrent à l'émergence du concept de « perturbation endocrinienne ». Les travaux de notre équipe (Tufts University School of Medicine, Boston, MA, USA) sur la présence d'oestrogène dans des produits plastiques apportèrent des éléments décisifs. Un domaine scientifique interdisciplinaire a émergé des concepts fondamentaux proposés à cette occasion. Le domaine émergeant de la biologie écologique du développement contestait l'idée selon laquelle le développement était un « programme ». Cela a créé une situation de dissonance théorique. Toutefois, la biologie écologique du développement a réussi à produire de nouveaux concepts et un ensemble de preuves documentant les effets délétères des perturbateurs endocriniens sur la santé. Au cours des années 2010, quelques mesures réglementaires ont été prises en Europe. Cette lenteur est une conséquence de l'inertie réglementaire, des lobbies qui entravent les efforts de réglementation, et des ambiguïtés théoriques qui pèsent sur les chercheurs et qui sont habilement exploitées par les lobbies. En 2012, la Société d'Endocrinologie a proposé que les principes de l'endocrinologie guident la recherche et la réglementation des perturbateurs endocriniens. Pour aller plus loin dans cette proposition raisonnable, nous proposons de creuser les bases théoriques de la biologie afin de dégager des principes théoriques solides et précis. En effet, selon Ludwig Boltzmann, « rien n'est plus pratique qu'une bonne théorie ». Cela s'applique non seulement à la conduite de la recherche, mais aussi au blocage efficace des effets pernicieux de ceux qui entravent la création d'une politique de santé protectrice en créant le doute et l'ignorance.
Mots-clés: Xénoestrogènes, Bisphénol-A, origines fœtales des maladies de l'adulte, héritage transgénérationnel, théorie biologique, organicisme, biologie écologique du développement (eco-devo)

The publication in 1962 of Rachel Carson's book *Silent Spring*[1] is considered a defining moment regarding public awareness of the deleterious consequences of human actions on the ecosystem. Carson examined the results of widespread pesticide use, triggered the development of environmental activism and led to the creation of the US-Environmental Protection Agency (USEPA) in 1970. Carson assumed cancer was a direct result of pesticide exposure but considered that it could also arise indirectly via liver alterations that led to increased circulating levels of estrogen. In 1979, almost two decades after Carson's pioneering work, John McLachlan, a Developmental Toxicologist and Head of the Developmental Endocrinology and Pharmacology Section at the National Institute of Environmental Health Sciences[2] (NIEHS), organized the first of a series of conferences entitled "Estrogens in the Environment" at Research Triangle Park, NC, USA. At this event, emerging problems caused by various environmental estrogenic pollutants, the widespread use of oral contraceptives and the discovery of the diethylstilbestrol syndrome [3] were discussed in great detail [4].

In the decade that followed the first "Estrogens in the Environment" conference, Dr. Theo Colborn, who was a Senior Fellow at the World Wildlife Fund and the Conservation Foundation and a Fellow at the W. Alton Jones Foundation[5] at the time, conducted a survey with her collaborators on the state of the environment in the Great Lakes area. They observed that young animals exhibited morphological and functional alterations that caused their premature death or abnormal development. These alterations included metabolic changes that manifested as 'wasting'; the animals were lethargic, lost their appetites, experienced weight loss and died prematurely. Dr. Colborn and her colleagues also observed thyroid and heart problems, abnormal metabolism of iron, male birds growing ovarian tissue, female birds growing excessive oviduct tissue, male fish that did not reach full sexual maturity, and hermaphroditism in fish. Birth defects and behavioural changes were also observed by the team [6]. Dr. Colborn conjectured that these damaging health effects were the consequence of the decrease in levels of environmental estrogenic chemicals such as dichloro-diphenyl-trichloroethane (DDT) and polychlorinated biphenyls (PCBs) following the introduction of EPA regulations such as the banning of these chemicals in the 1970s. If the effects Colborn et al. described were indeed due to the residual action of the banned chemicals, which only became apparent once the initial effects of these chemicals on mortality had ended, there was nothing else to do but wait for a further decrease in these levels.

To the contrary, an accident in our laboratory at that time revealed that there were xenoestrogens in the environment that were yet to be identified and regulated [7]; this showed that waiting for further lowering of the DDT and PCBs levels would not suffice to make these pathologies disappear. These events took place towards the end of 1987 in our research lab at Tufts University School of Medicine

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1 See Fred Rowe Davis' paper in this issue.

2 For more on the NIEHS and its history: https://www.niehs.nih.gov/about/history/index.cfm

3 The diethylstilbestrol syndrome resulted from treatment of pregnant women to prevent miscarriage and manifested as reproductive problems in their offspring. See the "The Wingspread Conference and the endocrine disruption concept" section later in this paper.

4 *Estrogens in the Environment*. Edited by McLachlan JA. New York, NY: Elsevier, 1980.

5 Theo Colborn is now considered as a pioneer of environmental health. Michael Lemonick, « Theo Colborn », http://content.time.com/time/specials/2007/article/0,28804,1663317_1663323_1669901,00.html ; in French, see Stéphane Foucart, « Theo Colborn, 1927-2014 », *Le Monde*, 22 déc. 2014
https://www.lemonde.fr/planete/article/2014/12/24/theo-colborn-1927-2014_4545871_3244.html

6 Colborn T, Liroff RA. Toxics in the Great Lakes. *EPA Journal* 16, n°6 (1990): 5-8.

7 Soto AM, Justicia H, Wray JW, Sonnenschein C. *p*-Nonyl-phenol: An estrogenic xenobiotic released from "modified" polystyrene. *Environmental Health Perspectives* 92 (1991): 167-73.

in Boston. Our work centered on exploring the basic question of why cells proliferated. The accident we are referring to provided a strong argument to assess the problem that Colborn and her collaborators described, and its implications for environmental and human health.

Discovery of "unregulated" estrogens in the environment

While working on the estrogen regulation of cell proliferation in breast epithelial MCF7 cells[8], we observed that estrogens did not directly stimulate cell proliferation in these cells. Instead, the key to this phenomenon was an inhibitor present in the serum [9]; estrogens affected cell proliferation by neutralizing this inhibitor rather than by directly stimulating cell proliferation. These results inspired us to postulate that all cells, both in unicellular and multicellular organisms, proliferate and move constitutively when in the presence of adequate amounts of nutrients [10]. Based on this principle, we proceeded to purify the blood-borne inhibitor [11].

During this purification process, the resulting fractions were being tested both in the presence and the absence of estrogen; the fractions containing inhibitory activity inhibited proliferation, while estrogen supplementation overrode the inhibition. Unexpectedly, cells from diverse breast epithelial estrogen-target cell lines proliferated maximally. It took us four months of systematic substitutions to track the origin of the contamination down to the plastic tubes where estrogen-free serum was being stored. After one year of additional work, we identified *p*-nonylphenol as the estrogenic contaminant [12]. Nonylphenol is an alkylphenol used in the synthesis of non-ionic detergents and antioxidants. As a consequence of reporting these findings of unintended hormonal activity in laboratory plasticware, we were invited to participate in the 1991 Wingspread Conference, held in Racine, Wisconsin, where the term endocrine disruptor (ED) was coined [13]. The piece of the puzzle we brought to the Wingspread Conference was evidence of xenoestrogens in the environment that were yet to be identified and regulated. For example, nonylphenol, used in the synthesis of non-ionic detergents, was found in rivers [14]. Humans are also directly exposed to these detergents through their use in spermicides, one example being the widely used nonoxynol-9. Additionally, other alkylphenols were found in fish in the Detroit River's Trenton Channel, located close to a chemical plant manufacturing alkylphenols. These animals were reported to contain 40 µg of p-tert-pentylphenol per gram of fat tissue, a concentration higher

8 The MCF7 cells are breast tumor cells that have been used in research laboratories since the 1970s.

9 Sonnenschein C, Soto AM, Michaelson CL. Human serum albumin shares the properties of Estrocolyone-I, the inhibitor of the proliferation of estrogen-target cells. *Journal of Steroid Biochemistry and Molecular Biology* 59 (1996): 147-54.

10 Soto AM, Sonnenschein C. Regulation of Cell Proliferation: The negative control perspective. *Annals of the New York Academy of Sciences* 628 (1991): 412-18; Sonnenschein C, Soto AM. *The Society of Cells: Cancer and Control of Cell Proliferation*. New York: Springer Verlag, 1999; Soto AM, Longo G, Montévil M, Sonnenschein C. The biological default state of cell proliferation with variation and motility, a fundamental principle for a Theory of Organisms. *Prog Biophys Mol Biol* 122, no. 1 (Oct 2016): 16-23.

11 Sonnenschein C, Soto AM, Michaelson CL. Human serum albumin shares the properties of Estrocolyone-I, the inhibitor of the proliferation of estrogen-target cells. *Journal of Steroid Biochemistry and Molecular Biology* 59 (1996): 147-54.

12 Soto AM, Justicia H, Wray JW, Sonnenschein C. *p*-Nonyl-phenol: An estrogenic xenobiotic released from "modified" polystyrene. *Environmental Health Perspectives* 92 (1991): 167-73.

13 *Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*, edited by Colborn T, Clement C. Princeton: Princeton Scientific Publishing, 1992; Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives* 101 (1993): 378-84.

14 Markey CM, Michaelson CL, Sonnenschein C, Soto AM. Alkylphenols and Bisphenol A as environmental estrogens. In *The Handbook of Environmental Chemistry Vol 3. Part L, Endocrine Disruptors - Part I.* Edited by Metzler M. Berlin Heidelberg: Springer Verlag, 2001.

than that found in the river sediment [15]. Thus, it became obvious that action was urgently required, rather than simply waiting for the regulated chemicals to decay.

The Wingspread Conference and the endocrine disruption concept

Dr. Colborn brought together a group of 21 scientists at the Wingspread Conference to discuss the observations she and her colleagues had made on the state of the environment in the Great Lakes area. The opening statement of the Wingspread Declaration asserted that "Many compounds introduced into the environment by human activity are capable of disrupting the endocrine system of animals, including fish, wildlife, and humans. Endocrine disruption can be profound because of the crucial role hormones play in controlling development" [16]. To support this statement, participants discussed the following issues:

1) The link between alterations described in wildlife and the syndrome caused by the synthetic estrogen diethylstilbestrol (DES). Daughters born to mothers who were given DES during pregnancy to prevent miscarriages presented with the very rare cancer called vaginal clear cell adenocarcinoma, various genital tract abnormalities, reduced fertility, abnormal pregnancies and altered immune responses. Comparable effects were found in wildlife and laboratory animals exposed to xenoestrogens. These findings suggested that humans were also at risk when exposed to the same environmental hazards as wildlife.

2) The importance of the fact that low doses of hormones can produce significant effects. In litter bearing animals such as rodents, the intrauterine positioning of fetuses has marked effects on anatomical, functional and behavioural outcomes later in life. The sex of the neighbouring fetuses resulted in small local differences in sex steroid levels during fetal life, thus revealing that small physiological variations of hormone levels during morphogenesis had measurable consequences in the adult phenotype [17]. It was deemed crucial to know the levels of exposure to hormonally active chemicals in the general population because we expected them to be lower than those affecting wildlife exposed to DDT or humans exposed occupationally to pesticides [18].

3) The type of research needed to fully understand the problem and to assess the effect on human populations. It should be noted that at that time, the toxicological tests used by regulatory agencies assessed acute toxicity, mutagenicity and carcinogenicity, endpoints that were likely to miss most EDs given that they are seldom mutagens and that they affect developmental end points that were not covered by these tests [19]. The conferees concluded that new methods to detect and measure these types of toxicants needed to be developed [20]. We therefore adapted the assay for the inhibitor in serum (mentioned above) to screen for suspected estrogenic substances and uncovered estrogenic activity in

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15 Shiraishi H, Carter DS, Hites RA. Identification and determination of tert-amylphenols in carp from the Trenton Channel of the Detroit River, Michigan, U.S.A. *Biomedical and Environmental Mass Spectrometry* 18 (1989): 478-83.

16 Bern HA, Blair P, Brasseur S *et al.* Wingspread Consensus Statement. In *Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*. Edited by Colborn T, Clement C. Princeton: Princeton Scientific Publishing, 1992.

17 Vom Saal FS. Triennial Reproduction Symposium: Environmental programming of reproduction during fetal life: Effects of intrauterine position and the endocrine disrupting chemical Bisphenol A. *J Anim Sci* 94, no. 7 (Jul 2016): 2722-36. https://dx.doi.org/10.2527/jas.2015-0211; Vandenberg, LN, Maffini MV, Wadia PR, et al. Exposure to environmentally relevant doses of the xenoestrogen Bisphenol-A alters development of the fetal mouse mammary gland. *Endocrinology* 148 (2007): 116-27.

18 Guzelian PS. Comparative toxicology of Chlorodecone (Kepone) in humans and experimental animals. *Annual Review of Pharmacological Toxicology* 22 (1982): 89-113.

19 Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans." *Environmental Health Perspectives* 101 (1993): 378-84.

20 Bern HA, Blair P, Brasseur S *et al.* Wingspread Consensus Statement. In *Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*. Edited by Colborn T, Clement C. Princeton: Princeton Scientific Publishing, 1992.

multiple environmental contaminants that are produced in large volumes [21]. This assay is now known as the E-SCREEN assay [22].

Defining endocrine disruption

Most of the hormonally active environmental chemicals discussed at Wingspread mimicked estrogens, yet the effects described in wildlife involved multiple endocrine alterations and developmental anomalies. The conferees discussed at length what to call the phenomena we were witnessing and the chemicals that induced them. We settled on "endocrine disruption" for the former and "endocrine disruptor" for the latter [23]. The word "disruption" was used to indicate that the phenomena involved irreversible effects that changed the trajectory of development. In this regard, they evoked effects produced by severe congenital hypothyroidism (cretinism), a condition that cannot be entirely corrected by postnatal administration of thyroid hormone. This complexity gave rise to various definitions of endocrine disruptors. We prefer the definition adopted by the Endocrine Society: "an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action"[24]. This definition has the virtue of brevity and the advantage of not conflating the concept of interfering with hormone action with that of producing adverse effects.

The main conceptual themes examined at the Wingspread Conference

The main concepts mentioned in the Wingspread statement entered the scientific literature two years after the Wingspread Conference in an article by Colborn et al.[25]. Most of the foundational concepts for endocrine disruption were already accepted and current in endocrinology. The reliability of these concepts is demonstrated by almost three decades of peer-reviewed research publications since Wingspread. However, the theoretical paucity of biology made – and continues to make – it difficult to integrate these concepts into a detailed theoretical framework. In this regard, the Endocrine Society published a statement of principles of endocrinology that should frame research on the complex effects of EDs, and how they are interpreted[26].

The relative specificity of hormones and endocrine disruptors. The specificity of natural hormones is not a molecular property of the hormone; it actually depends on the evolutionary and developmental history of the target organs and their cells. For instance, high androgen doses produce a positive effect on the uterotropic assay, a test traditionally used to determine whether a compound has estrogenic properties[27]. Regarding EDs, bisphenol-A (BPA) is an estrogen agonist which interferes

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21 Soto AM, Chung KL, Sonnenschein C. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen sensitive cells. *Environmental Health Perspectives* 102 (1994): 380-83.

22 Soto AM, Sonnenschein C, Chung KL *et al*. The E-SCREEN assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. *Environmental Health Perspectives* 103 (1995): 113-22.

23 Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives* 101 (1993): 378-84.

24 Zoeller RT, Brown TR, Doan L *et al.* Endocrine-disrupting chemicals and public health protection: A Statement of Principles from the Endocrine Society. *Endocrinology* 153 (2012): 4097-110.

25 Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives* 101 (1993): 378-84.

26 Zoeller RT, Brown TR, Doan L *et al*. Endocrine-disrupting..., *op. cit.*

27 Armstrong DT, Moon YS, Leung PCK. Uterotrophic effects of testosterone and 5α-Dihydrotestosterone in intact and ovariectomized immature female rats. *Biology of Reproduction* 15 (1976): 107-14.

with thyroid hormone at higher doses[28]. Additionally, for some estrogen receptor-mediated endpoints, the effects of estradiol and BPA are different, and sometimes opposite[29].

Organizational versus activational effects. EDs may affect health at any and all stages. However, the effects of exposure during embryonic and fetal development are generally more devastating during organogenesis; these so-called "organizational" effects are mostly irreversible [30]. Activational effects seem to be mostly reversible and disappear once exposure ceases. However, not all exposures during adulthood are activational[31]. For instance, female mice exposed to low doses of BPA during pregnancy develop glucose intolerance and altered insulin sensitivity several months after delivery. In this case, unlike activational effects, the deleterious effects become detectable several months after cessation of exposure[32].

Because the endocrine system regulates multiple functions including growth, development and metabolism, it is expected that the syndromes produced by ED exposure will be complex and include direct and indirect effects. For instance, some EDs increase the risk of obesity; this condition affects pubertal timing, and consequently, it may indirectly increase the risk of breast cancer [33]. Additionally, EDs may also alter the whole hormonal milieu, and thus simultaneously affect many reproductive tissues[34]. Although experimental and epidemiological studies often investigate a single chemical at a time, the combined effect of these chemicals is of great relevance, since humans are usually exposed simultaneously to a multitude of EDs[35].

Dose duration and timing of exposure: historicity and contextuality

History is irrelevant in physics, as the field's constants emerged soon after the Big Bang and physical objects have not changed since then. However, biological objects are relentlessly changing, both during ontogeny and phylogeny. Moreover, organisms exist in the dual context of their internal milieu and the environment that they inhabit. These two characteristics must always be present when assessing exposures to environmental chemicals such as EDs.

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28 Zoeller RT, Bansal R, Parris C. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters Rc3/Neurogranin expression in the developing rat brain. *Endocrinology* 146 (2005): 607-12.

29 Kurian JR, Keen KL, Kenealy BP, et al. Acute influences of Bisphenol A exposure on hypothalamic release of Gonadotropin-Releasing Hormone and Kisspeptin in female Rhesus monkeys. *Endocrinology* 156, no. 7 (Jul 2015): 2563-70; Speroni L, Voutilainen M, Mikkola ML, et al. New insights into fetal mammary gland morphogenesis: Differential effects of natural and environmental estrogens. *Sci Rep* 7 (Jan 19 2017): 40806; Camacho L, Lewis SM, Vanlandingham MM, et al. A two-year toxicology study of Bisphenol A (BPA) in Sprague-Dawley rats: CLARITY-BPA core study results. *Food Chem Toxicol* 132 (Oct 2019): 110728; Nadal A, Fuentes E, Ripoll C, et al. Extranuclear-initiated estrogenic actions of endocrine disrupting chemicals: Is there toxicology beyond paracelsus?, *J Steroid Biochem Mol Biol* 176 (Feb 2018): 16-22.

30 Phoenix CH, Goy RW, Gerall AA, Young WC. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology* 65 (1959): 369-82.

31 Guzelian PS. Comparative toxicology of chlorodecone (Kepone) in humans and experimental animals. *Annual Review of Pharmacological Toxicology* 22 (1982): 89-113.

32 Alonso-Magdalena P, Garcia-Arevalo M, Quesada I, Nadal A. Bisphenol-A treatment during pregnancy in mice: A new window of susceptibility for the development of diabetes in mothers later in life. *Endocrinology* 156, no. 5 (2015): 1659-70.

33 Gore AC, Chappell VA, Fenton SE *et al.* EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. *Endocr Rev* 36, no. 6 (Dec 2015): E1-e150.

34 Sonnenschein C, Wadia PR, Rubin BS, Soto AM. Cancer as development gone awry: The case for Bisphenol-A as a carcinogen. *Journal of Developmental Origins of Health and Disease* 2 (2011): 9-16.

35 Kortenkamp A, Faust M, Scholze M, Backhaus T. Low-level exposure to multiple chemicals: Reason for human health concerns?, *Environmental Health Perspectives* 115 Suppl 1:106-14. (2007): 106-14.

Dose: Exposure to xenoestrogens during fetal development produced effects at much lower doses [36] than those required *in vitro* or in the standard toxicological test, i.e., the uterotropic assay. Various low-dose effects have now been clearly mapped to extranuclear receptors such as ER alpha, beta and GPR30 [37].

Nonmonotonicity: Even before Wingspread, it was well established that hormones often exhibit non-monotonic dose-response curves (NMDRC), namely, nonlinear relationships between dose and effect which are characterized by a change in the sign (positive/negative) of the slope of the curve over the range of doses examined. For example, the proliferative effect of estrogens [38] and androgens is biphasic; at lower doses, the net effect results in more cells, while higher doses lead to a lower cell number [39]. These two effects are mediated by distinct processes that can be separated from each other in an experimental setting [40]: the NMDRC is a composite of two or more monotonic curves. Not surprisingly, EDs also exhibit NMDRCs [41]. For example, BPA and bisphenol-S are potent estrogens when acting via extranuclear ERα, ERβ and GPER, and the NMDRC they elicit in pancreatic ß cells could be attributed to the fact that the different components of the curve are mediated by distinct pathways involving different receptors [42].

Duration of the hormonal stimulus and time-frame of exposure: Hormones produce dissimilar effects depending on whether they are administered in a single dose (acute effect) or continuously (chronic effect). For example, a single estrogen dose induces a wave of cell proliferation on the luminal epithelium of the uterus of ovariectomized rodents, while continuous administration first induces proliferation and later inhibits further proliferation [43]. A similar phenomenon applies to the effect of androgens in the prostate [44]. Moreover, the same hormone can produce dissimilar and even contrary effects in different targets, or in the same target, over different time frames [45]. Additionally, a hormone may affect different tissues when administered to animals during different developmental stages [46].

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36 Rubin BS, Lenkowski JR, Schaeberle CM *et al.* Evidence of altered brain sexual differentiation in mice exposed perinatally to low environmentally relevant levels of Bisphenol A. *Endocrinology* 147 (2006): 3681-91.

37 Nadal A, Fuentes E, Ripoll C, et al. Extranuclear-initiated estrogenic actions of endocrine disrupting chemicals: Is there toxicology beyond paracelsus?, *J Steroid Biochem Mol Biol* 176 (Feb 2018): 16-22.

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40 Sonnenschein C, Olea N, Pasanen ME, Soto AM. Negative controls of cell proliferation: Human prostate cancer cells and androgens. *Cancer Research* 49 (1989): 3474-81; Geck P, Maffini MV, Szelei J, et al. Androgen-induced proliferative quiescence in prostate cancer: The role of AS3 as its mediator. *Proceedings of the National Academy of Science of the United States of America* 97 (2000): 10185-90; Soto AM, Lin TM, Sakabe K, et al. Variants of the human prostate LNCaP cell line as a tool to study discrete components of the androgen-mediated proliferative response. *Oncology Research* 7 (1995): 545-58.

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42 Nadal A, Fuentes E, Ripoll C, et al. Extranuclear-initiated estrogenic actions of endocrine disrupting chemicals: Is there toxicology beyond paracelsus?, *J Steroid Biochem Mol Biol* 176 (Feb 2018): 16-22.

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45 Soto AM, Sonnenschein C. The two faces of Janus: Sex steroids as mediators of both cell proliferation and cell death. *Journal of the National Cancer Institute* 93 (2001): 1673-75.

46 Kang YH, Anderson WA, De Sombre ER. Modulation of uterine morphology and growth by estradiol-17 beta and an estrogen antagonist. *Journal of Cell Biology* 64 (1975): 682-91; Martin L, Finn CA, Trinder G. Hypertrophy and

Another important factor is the velocity of changes in the hormone level. A rapid increase of estradiol levels during the pre-ovulatory period triggers a positive feedback response and LH release leading to ovulation [47], while constant, low estrogen levels generate a negative feedback [48]. In sum, the historicity of the organism and the contextuality of the response to hormones should be taken into consideration when evaluating the effect of EDs. The effect is not expected to be the same when different exposure ages, exposure durations and types of exposure (acute, chronic, constant, gradient, pulsatile, etc.) are used.

New developments after the 1991 Wingspread Declaration

The concepts at the core of the Wingspread declaration were adopted by researchers working in this new field, rather than using the high exposure levels that are common in toxicology; these scientists used low doses and the assumption of nonmonotonicity. The majority of these researchers are endocrinologists and developmental and reproductive biologists. Among a number of important findings, several were of immediate concern for public health. For example, the effects of endocrine disruptors occurred at low exposure levels within the range of human exposure and, like natural hormones, EDs often displayed nonmonotonic dose-response curves.

One of these new findings was that developmental exposure to BPA in rodents causes a complex array of effects that resemble those observed after developmental exposure to DES, including the development of mammary/breast cancer and deleterious reproductive effects. Fetal and neonatal exposure to environmentally relevant doses of BPA induces both earlier vaginal opening and earlier first estrus, alters estrous cyclicity and induces early cessation of cyclic activity. Exposed animals also show decreased fertility and fecundity [49]. In the mammary gland, BPA induces preneoplastic lesions in mice [50], and carcinoma *in situ* [51] and palpable tumors [52] in rats. The similar effects observed in DES and BPA fetal exposure lead us to conclude that it is reasonable to infer that the BPA effects in rodents are predictive of effects in humans. Moreover, it was found that low-level fetal exposure to BPA resulted in metabolic syndrome, obesity and altered behaviors in both male and female rodents. All these effects of BPA resemble pathologies that are on the rise in human populations [53]. Additionally,

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hyperplasia in the mouse uterus after oestrogen treatment: An autoradiographic study. *Journal of Endocrinology* 56 (1973): 133-44.

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a concerning new phenomenon of transgenerational effects of BPA and other endocrine disruptors has been observed. Transgenerational effects are defined as those occurring as a result of fetal exposure of the grandparent generation, yet manifested in grandchildren and beyond [54].

All these discoveries were made at a time when certain widely held ideas (such as that of development being a result of a genetic program) were being contested and new scientific views were proposed which considered the environment as a co-determinant of the phenotype. This situation needs to be addressed because it leads us to the crucial issue of the best fitting theoretical framework for endocrine disruption. Theories are practical tools to gather knowledge and work best when they are precise. Moreover, when we are addressing a scientific issue with practical consequences to human and environmental health, like EDs, regulatory decisions can easily be postponed by challenging the nature of the supporting evidence presented. This evidence depends on whether the theory used is vague or rigorous.

Theoretical challenges

The pioneers of the molecular biology revolution fostered the idea that biology could be reduced to chemistry and physics. Some evolutionary biologists resisted this reductionist turn and predicated the autonomy of biology as a science and also the importance of theories. Despite this resistance, the idea that scientists have direct access to the objects they wish to study gradually gained traction, and eventually terms like "information", "program" and "signal" were reified. The aforementioned molecular biologists considered this reification as evidence that scientists can directly observe reality, a naïve and counterproductive idea [55]. Indeed, a main characteristic of the natural sciences (including biology before the molecular biology revolution) is that the ideas and methods put forward by scientists are counterintuitive and usually contrary to common sense [56]. The frame of reference we use as scientists is thus different to that we all use in everyday ordinary situations. For example, in common language people talk about sunrise and sunset, despite having learned during childhood about the heliocentric planetary system. This example also illustrates why the naïve perception that facts can exist independently of any reference frame is incorrect. There is no observation devoid of theoretical content; sunrise and sunset refer to the sun rotating around the earth, as in Ptolemy's theory. As put by the philosopher DC Dennett: "There is no such thing as philosophy-free science; there is only science whose philosophical baggage is taken on board without examination" [57].

The role of theories: Within this context, scientists purposely suspend the common-sense world view used by all in our everyday lives when constructing theories and testing them with experiments. Scientific theories provide organizing principles and construct objectivity by framing observations and experiments. Even research performed within the frame of a "wrong" theory sooner or later will result in the demise of such a theory, thus advancing our knowledge. This process requires that the theory in

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Kandarakis, E., J. P. Bourguignon, Giudice LC *et al.* Endocrine-disrupting chemicals: An Endocrine Society scientific statement. *Endocrine Reviews* 30 (2009): 293-342.

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57 Dennett DC. *Darwin's Dangerous Idea*. New York, NY: Simon & Schuster, 1995.

question has clear enunciates that allow for their demise through both theoretical and experimental considerations. A comment by the mathematician and physicist Henri Poincaré, published before the dismissal of the ether theory, illustrates this point: "Whether the ether exists or not matters little-- let us leave that to the metaphysicians; what is essential for us is, that everything happens as if it existed, and that this hypothesis is found to be suitable for the explanation of phenomena. After all, have we any other reason for believing in the existence of material objects? That, too, is only a convenient hypothesis; only, it will never cease to be so, while some day, no doubt, the ether will be thrown aside as useless" [58]. Indeed, the "luminiferous ether theory" ceased to be useful at the beginning of the 20th century. Light was found to have both wave and particle properties; particles do not need a medium to travel.

Theoretical inconsistencies and their exploitation by those that oppose regulation

The birth of the endocrine disruptor concept was simultaneous with important theoretical developments in the biological sciences. By then, it had become obvious that the molecular biology revolution had failed to fulfill its predictions; neither cells nor organisms can be equated with computers. They cannot be programmed or reprogrammed [59]. Philosophers and theoreticians repeatedly warned of the inadequacy of these metaphors [60]. The ascent of the disciplines known as "ecological developmental biology (eco-devo)" and evolutionary developmental (evo-devo) biology also brought a critique of the genocentric views in biology [61]. Not only did eco-devo bring to light the role of the environment as a co-maker of phenotypes, but in doing so, it explicitly rejected the idea of a genetic developmental program, or the supremacy of genetic explanations over functional ones. In other words, genes do not hold a privileged causal role; instead, there is redundancy and plasticity.

Another important development that coincided with the birth of the endocrine disruptor field is the ascent of organicism. Organicism has its philosophical bases in Immanuel Kant and in his vision of the organism. The organicist school emerged between the two World Wars in continental Europe, Great Britain and the US. Its proponents rejected the traditional opposing views of reductionism and vitalism and aimed to create a third way that circumvented the limitations of both. They considered organisms as organized systems, rather than an aggregate that can be reduced to physics or chemistry. They therefore believed that biology was an autonomous discipline that needed its own theories [62]. Moreover, these biologists made it clear that mechanism was not an adequate type of explanation because in biology, explanations address the reciprocal relations between parts and whole in living systems. Alternative ways therefore had to be constructed to explore causality[63]. In this circular organization regime, the parts depend on the whole and vice versa; this organizational regime not only produces and maintains the parts that contribute to the functioning of the whole integrated system, but the integrated system also interacts with its environment to promote the conditions of its own existence. This contrasts with the intrinsically reductionist stance still dominant in biology and the belief that the only licit explanation in biology is through molecular mechanisms.

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58 Poincaré H. *Science and Hypothesis*. London: Walter Scott Publishing Co, 1905.

59 Soto AM, Sonnenschein C. Reductionism, organicism, and causality in the biomedical sciences: A critique. *Perspect Biol Med* 61, n° 4 (2018): 489-502.

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An additional development has been taking place in evolutionary biology since the advent of endocrine disruption. Due to the strong theoretical frame of this discipline, the introduction of novel concepts such as the role of epigenetic inheritance has generated wide range discussions about what concepts should be kept, which of them require modification, and which should be rejected [64]. In contrast, the lack of theoretical engagement by the mainstream in organismal biology leads to the co-existence of conflicting postulates, whereby lacks of fit between data and presuppositions are easily fixed by *ad hoc* additions. For example, the role of the environment as a determinant of phenotype is metaphorically referred to as "reprogramming", even by those who are aware that development is not a "program". Rather than referring to "programming" and "reprogramming", it would be preferable to use the more neutral term "environmentally elicited" or "the induction of developmental plasticity"[65].

Although frequently ignored by biologists, as we mentioned above, philosophical stances matter. Thus, to the delight of those who would like to delay regulatory action regarding EDs, some biologists have proposed developing "adverse effect pathways" [66] that would connect molecules to adverse effects through "mechanisms". They are attempting to implement this approach while theoretical biologists are questioning whether mechanisms play an explanatory role in organismal phenomena. Indeed, the search for molecular mechanisms seldom leads to the discovery of a linear causal chain, since the structure of determination of complex biological systems is hardly ever linear. This pragmatic problem, namely that regulation of the use of these chemicals is systematically transferred to an indeterminate future, motivated a group of researchers to look for an alternative solution with immediate practical consequences. It consists of putting mechanism aside and instead using key characteristics (KC) as the basis for hazard identification, namely the "common features of hormone regulation and action that are independent of the diversity of the effects of hormones during the life cycle". Additionally, "KCs of EDCs are the functional properties of agents that alter hormone action", and "… KCs are agnostic with respect to current or future knowledge of downstream health hazards and mechanistic pathways" [67]. Although the proponents of this alternative do not offer theoretical arguments on the validity of mechanistic pathways, their approach has the virtue of not depending on stances of dubious validity, and most importantly, could be immediately applied for rigorous regulatory purposes. This pragmatic solution does not make the resolution of theoretical issues superfluous or less timely.

Another hindrance to regulation is the resistance of regulatory organisms like the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) to consider the validity of nonmonotonic dose-response curves (NMDRC). When these regulators deal with linear (monotonic) responses, no knowledge of the alleged molecular mechanisms underlying each one of these NMDRC is needed to recognize the existence of this well-described statistically significant phenomenon [68]; the lack of a mechanistic explanation should not therefore be used to dismiss NMDRC obtained with statistically appropriate methods in regulatory science [69]. As nonmonotonic curves cover a wide range of shapes, it is not a good practice to try to fit the data to a few arbitrary models on a few quantities,

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68 Soto AM, Sonnenschein C. Endocrine disruptors - Putting the mechanistic cart before the phenomenological horse. *Nat Rev Endocrinol* 14, no. 6 (2018): 317-18; Zoeller RT, Vandenberg LN. Assessing dose-response relationships for endocrine disrupting chemicals (EDCs): A focus on non-monotonicity. *Environ Health* 14 (2015): 42.

69 Montévil M, Acevedo N, Schaeberle CM *et al*. A combined morphometric and statistical approach to assess nonmonotonicity in the developing mammary gland of rats in the CLARITY-BPA study. *Environ Health Perspect* 128, no. 5 (May 2020): 57001.

and thus easily dismiss data and interpretations that go beyond these arbitrary models [70]. Instead, a more complete analysis based on mathematical considerations is needed to do justice to observations [71]. Regulatory agencies would benefit from introducing more appropriate mathematical structures and statistical treatments into their analysis than those they arbitrarily use today. This adjustment requires the involvement of independent scientists who are well versed in advanced mathematics and statistics.

Conclusions

The conceptual developments brought about by eco- and evo- devo have been helpful in providing a loose theoretical framework that allowed scientists who study endocrine disruption to circumvent hindrances resulting from misused program, information and mechanism ideas. However, a more precise theoretical framework is urgently needed to counteract the spurious arguments put forward by regulators and some academic scientists regarding the role of mechanisms and of NMDRC in regulatory science. The organicist stance provides the conceptual bases for the elaboration of solid principles for a theory of organisms [72] that spans the complete lifecycle. These principles should certainly provide an adequate framework for experimentation and also help to exclude unreasonable regulation-hindering arguments from so-called regulatory science.

Theories have a practical purpose, namely, to construct objectivity and to determine what can be observed [73]. When a theory is not vague, it also permits us to decide when to reject an interpretation or drop a hypothesis. Our theoretical work in endocrine disruptors, fetal origins of adult diseases and carcinogenesis allowed us to deal with this type of problem and restrained us from looking for ad-hoc explanations, or from using terms loaded with theoretical baggage that, when made explicit, are incompatible with the accepted theoretical framework [74]. This positive experience has convinced us to propose a future direction for the field of endocrine disruption, namely the construction of a rigorous and explicit theoretical framework. This work will not only facilitate research by identifying the proper postulates and observables, but may serve to unmask, and even overcome, spurious arguments intended to delay regulation.

In brief, sufficient data have been gathered on the deleterious effects of EDs to warrant immediate action to decrease human exposure to these agents by means of a carefully planned and enforceable public health policy. Fortunately, in the particular case of BPA, the enormous body of evidence gathered mostly through studies in academia resulted in BPA being listed by the European Chemicals Agency (ECHA) as an ED that impacts human health. BPA is now also listed in the Candidate List of substances of very high concern (SVHCs) due to its reproductive toxicity properties. More recently, the European Commission published a chemicals strategy for sustainability. It proposes "to establish legally binding hazard identification of endocrine disruptors, based on the definition of the WHO, building on criteria already developed for pesticides and biocides, and apply it across all legislation;

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70 https://www.efsa.europa.eu/en/news/bisphenol-efsa-draft-opinion-proposes-lowering-tolerable-daily-intake

71 Montévil M, Acevedo N, Schaeberle CM *et al.* A combined morphometric and statistical approach to assess nonmonotonicity in the developing mammary gland of rats in the CLARITY-BPA study. *Environ Health Perspect* 128, no. 5 (May 2020): 57001.

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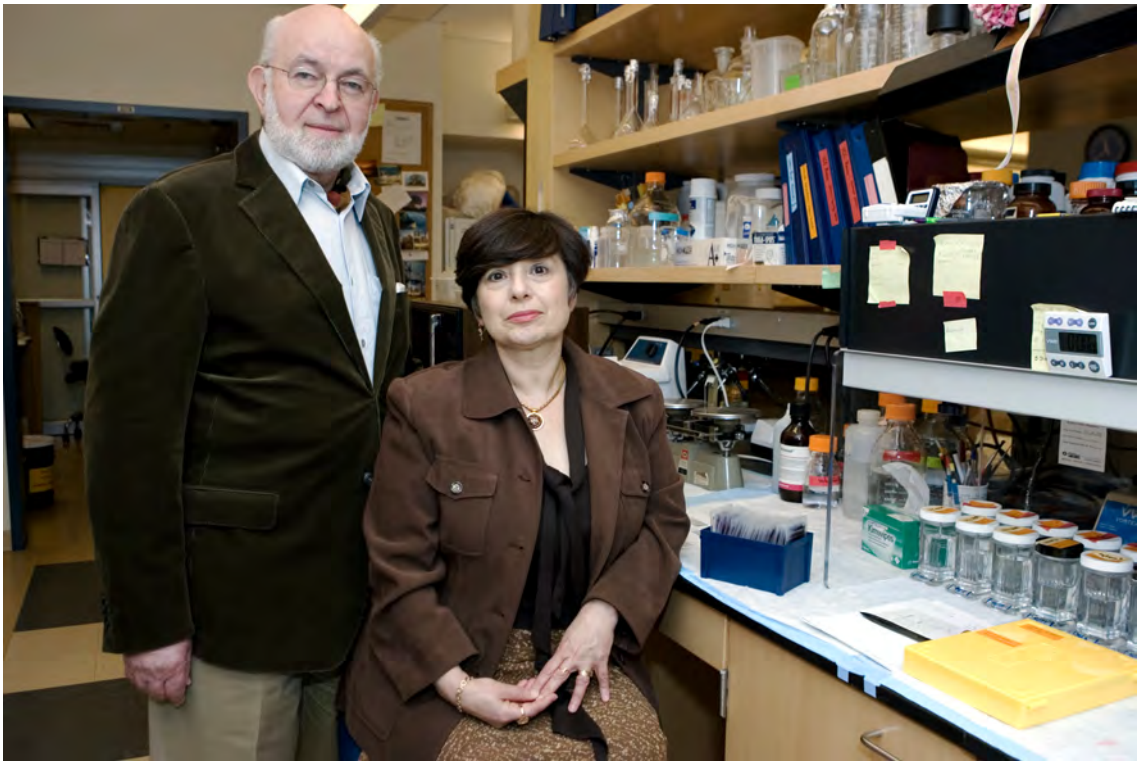
ensure that endocrine disruptors are banned in consumer products as soon as they are identified" [75]. This great step forward is the result of the efforts of scientists, journalists, politicians and medical and scientific societies. Endocrinologists should be ready to counter the usual delaying tactics of entities that oppose regulation and be armed with appropriate and rigorous principles and a clear understanding of when "enough is enough". In order to speed up the regulatory process, it will be necessary, on the one hand, to perform regulatory science informed by sound theoretical principles, and on the other, to use the precautionary principle as a guide for a protective public and environmental health policy.



Carlos Sonnenschein and Ana Soto, in their laboratory at Tufts University, personal collection

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75 European Commission. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, October 14, 2020. https://ec.europa.eu/environment/pdf/chemicals/2020/10/Strategy.pdf