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Regulatory Decisions on Endocrine Disrupting Chemicals Should be Based on the Principles of Endocrinology

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Abstract

For years, scientists from various disciplines have studied the effects of endocrine disrupting chemicals (EDCs) on the health and wellbeing of humans and wildlife. Some studies have specifically focused on the effects of low doses, i.e. those in the range that are thought to be safe

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for humans and/or animals. Others have focused on the existence of non-monotonic dose-response curves. These concepts challenge the way that chemical risk assessment is performed for EDCs. Continued discussions have clarified exactly what controversies and challenges remain. We address several of these issues, including why the study and regulation of EDCs should incorporate endocrine principles; what level of consensus there is for low dose effects; challenges to our understanding of non-monotonicity; and whether EDCs have been demonstrated to produce adverse effects. This discussion should result in a better understanding of these issues, and allow for additional dialogue on their impact on risk assessment.

Keywords

weight of evidence; organizational; adaptive effect; hormesis; human exposure; epidemiology; flare

1. Introduction

Even before the term “endocrine disruptor” was coined in 1991, toxicologists, endocrinologists, epidemiologists and others studied chemicals with endocrine disrupting properties, now known as endocrine disrupting chemicals (EDCs), with the intent of understanding whether these substances impact human and/or wildlife health. Over the past two decades, a large volume of published data has been produced and synthesized, with a more recent emphasis on studies that examine environmentally relevant exposures. In 2009, the German Federal Institute for Risk Assessment organized a meeting to address the scientific evidence that EDCs, and in particular pesticides used on crops, could have effects on human health. During that meeting, “concern was raised that effects due to non-monotonic dose-response curves might be overlooked in current guideline-conform[ing] toxicity testing for which doses are employed that typically exceed human exposure by orders of magnitude” [1]. It was clear from discussions at that meeting that there was no general consensus amongst academic scientists, regulators and industry scientists over use of the term “low dose” or whether “low dose effects” or non-monotonic effects had been generally established for EDCs. Following that meeting, we assembled a group of twelve scientists and spent more than two years reviewing hundreds of publications to determine the state of the science of low dose effects and non-monotonic dose responses for EDCs [2].

In our review, we addressed the “low dose paradigm”, which postulates that EDCs have effects on exposed animals at doses that are thought to be safe for humans, and that humans are also affected by environmentally relevant doses. Although this phenomenon was once referred to as a “hypothesis” [3], there is sufficient data to advance it beyond the hypothetical. Importantly, data supportive of the low dose paradigm were examined and analyzed more than a decade ago by an expert panel assembled by the National Toxicology Program (NTP) at the behest of the US Environmental Protection Agency (EPA). In a 2002 publication, the NTP panel defined low dose effects and the low dose cut-off as “biologic changes that occur in the range of human exposures or at doses lower than those typically used in the standard testing paradigm of the US EPA for evaluating reproductive and developmental toxicity” [4]. The panel identified low dose effects for four EDCs including the effects of genistein on brain sexual dimorphisms, male mammary gland morphology, and immune responses; the effects of diethylstilbestrol (DES) on prostate weight; the effects of methoxychlor on the immune system; and the effects of nonylphenol on brain sexual dimorphisms, thymus weight, estrus cyclicity and immune responses. Further, the NTP panel noted that the low dose effects they identified had dose response curves that “may be low-dose linear, threshold-appearing, or nonmonotonic” [4]. These conclusions were drawn

from as few as three studies per chemical, some of which had not yet been peer-reviewed [5].

Our more recent analysis was designed to take the conclusions from the NTP panel further, to ask whether low dose effects are a more general phenomenon that could be attributed to EDCs, or whether they are limited to this small number of chemicals identified by the NTP. Our efforts were hampered somewhat by the paucity of information on the range of typical human exposures and doses used in toxicological testing for many EDCs; however, we found evidence for low dose effects for more than two dozen EDCs [2].

We used a weight-of-evidence (WoE) approach to specifically focus on a few examples of low dose effects that we felt had a sufficiently large number of studies. Our WoE analyses included the effects of atrazine on sexual differentiation in male amphibians, the effects of BPA on the prostate and mammary gland, the effects of dioxin on spermatogenesis, and the effects of perchlorate on thyroid hormone levels in humans [2]. Yet it should be noted that there were several examples we identified but did not analyze in detail, including the effects of PCB mixtures on brain sexual dimorphisms [6–8], the effects of DDT on neurobehaviors [9–15], the effects of nicotine on human male genital tract malformations [16–19], the effects of octylphenol on the testes [20–23], and the effects of tributyltin on obesity [24–27], among others.

Our review also addressed the question of whether non-monotonic dose response curves (NMDRCs) were common in the EDC literature. NMDRCs are a relatively common feature in the field of endocrinology, and have been observed for many hormones on many different endpoints [28–30]. Further, NMDRCs are commonly reported in pharmacology in response to treatment with drugs and chemicals that interact with receptors [31–33]. Yet for years there has been controversy about NMDRCs from EDC exposures [34–36], including suggestions that NMDRCs could be specific to only a few EDCs – and therefore without broader relevance. To address this debate, we examined the peer-reviewed literature using search terms including “non-monotonic”, “U-shaped”, and “biphasic” responses. We also specifically searched for all peer-reviewed studies for specific EDCs identified in the FDA’s endocrine disruptor database [37]. We found examples of NMDRCs in cultured cells, in laboratory animals, and even in human populations for more than 70 EDCs from a range of chemical classes [2].

Following the publication of our analysis, an editorial was written by the director of the National Institutes of Environmental Health Sciences, Dr. Linda Birnbaum, who stated that our analysis provided “important insight into the effects of environmental chemicals on health-related end points and [addressed] the mechanistic questions of how chemicals with hormonal activity can have effects at external doses that are often considered safe by the regulatory community” [38]. More importantly, Birnbaum noted that “the question is no longer whether nonmonotonic dose responses are ‘real’ and occur frequently enough to be a concern; clearly these are common phenomena with well-understood mechanisms. Instead, the question is which dose–response shapes should be expected for specific environmental chemicals and under what specific circumstances.”

Since the NTP’s expert panel released its analysis of low dose effects, there have been rebuttals to the low dose paradigm [3, 39, 40]. A recent paper, written in response to our review, concludes that “the case for widespread nonmonotonicity leading to undetected toxicity at low doses has not been made” [41]. Unfortunately, this recent review draws conclusions from a total of fourteen citations (compared to the more than 800 we reviewed), and contains several incorrect or misleading statements (see Table 1). Here, we address some of the controversial issues raised in our review that have been contested by Rhomberg

and Goodman [41] and others [34, 35]. We expect that a better understanding of these issues as well as our approach to analyzing the EDC literature will allow for future conversations in a more collaborative and constructive vein.

2.1 Issue #1: Why should the study of EDCs rely on endocrine principles?

We and others have proposed that the study of EDCs, as well as the regulation of these chemicals by risk assessment agencies, should take into account several major principles of Endocrinology [2, 42–44]. Natural hormones are known to follow certain biological rules, and it therefore follows that chemicals that mimic or block the actions of hormones would also follow similar rules. Below, we discuss five major principles that must shape the conversation about EDCs and whether they are safe in their various current uses. We expect that this discussion of Endocrine Principles will allow for a scientifically-focused discussion of the science of endocrine disruption, and how the study of EDCs will continue to benefit from advances in environmental health science, which operates at the intersection of multiple fields including ecology, endocrinology, and toxicology [45].

Principle 1: Hormones are responsible for the health and well-being of animals (including humans) from the time of conception until death and control and integrate the systems of the body

There is virtually no organ or system in the body that is not controlled in some way by the endocrine system. Thus, while the hormone estrogen is often studied for its effects on the female reproductive system and secondary sex characteristics, estrogens are also responsible for maintaining male reproductive health, bone density, cardiovascular health, and the immune system, among others [46, 47]. Further, the role that hormones have during adulthood can be completely different from the roles of the same hormone during early development. For example, thyroid hormone is thought of as a thermostat for the body, i.e. as the control system for the metabolic machinery as well as bone health [48]. But during early development, thyroid hormone is essential for proper brain development [49]. Changes in the thyroid hormone signaling pathway lead to severe neural deficiencies including mental retardation, and the severity of these developmental defects is dependent on the timing of thyroid hormone deprivation [50].

Principle 2: The effects of hormones are mediated by highly specific interactions with receptors and the response is context dependent

Hundreds of hormones circulate throughout the body. These include the well-studied sex hormones estrogen and testosterone, thyroid hormone, hormones released by the pituitary (i.e. TSH, FSH, LH, prolactin, etc.) and the hypothalamus (i.e. TRH, GHRH, GnRH, etc.), hormones secreted by the digestive tract and its associated organs (i.e. ghrelin, IGF, angiotensin, insulin, glucagon, etc.), hormones from the adrenals (i.e. cortisol, adrenaline, dopamine, etc.) and more. The ability of cells and tissues to respond to hormones is dependent on the affinity of the hormone for the receptor (which can vary within a family of hormones such as the natural estrogens [51]), the number of receptors, the concentration of the hormone, and the presence of various co-factors that can enhance or repress the effects of the hormone [52, 53].

Because of the specificity of the hormone-receptor interactions, hormones can only act where receptors are present. Each of these signaling molecules circulates in the body but has very precise effects because the receptor-hormone interactions are highly specific [54]. The expression profiles of the receptors change throughout development, therefore hormones can have actions in one tissue at one time, and no action in the same tissue at another time. For example, genetic reporter mice designed to show where thyroid hormone receptor is

activated indicate that thyroid hormone action is required for the development of the metencephalic and diencephalic vesicles and the ganglia of the cranial nerves on embryonic day (E)11.5 [55]. One day later, thyroid hormone receptor is also expressed in the developing eye. Thus, deficiencies in thyroid hormone that are limited to E11.5 would have no effect on the eye, but deficiencies at E12.5 would be detrimental to this organ.

Principle 3: Hormones act at low doses

It is well established that most circulating hormones are found in the body in the part-per-trillion or part-per-billion concentration range [56]. In spite of these low concentrations, hormones have extremely potent effects because of the strong binding affinities they exhibit to their receptors, and because the binding of a hormone to a receptor leads to an amplified response in target cells. Further, because the relationship between hormone concentration and receptor occupancy is not linear, an increase in hormone concentration in the low dose range produces a large change in the percent of occupied receptors, whereas the same increase in concentration in the upper range produces a small change in the percent of occupied receptors [29]. Similarly, there is a non-linear relationship between receptor occupancy and biological effect, thus even small changes in the low dose range can produce large changes in biological endpoints [29, 57]. In most biological systems, fewer than 5% of all receptors are bound, thus the system is ‘primed’ to have large effects from relatively small changes in hormone concentration [30].

Principle 4: Hormones can exhibit non-linear, and often non-monotonic, dose response curves

Hormones have been shown to produce NMDRCs in cells, tissues and organs and their mechanisms have been well-characterized by endocrinologists. These curves manifest at different places along the dose response curve, depending on the endpoint being assessed. In one of the more common observed mechanisms, low doses of hormones produce increases in cell number, but at higher doses fewer cells are present, leading to an inverted U-shaped curve see for example [29, 58–60]). These relationships can be observed when two overlapping monotonic curves with opposing biological effects are present. In some cases, this occurs because high concentrations of the hormone are cytotoxic [29]. Yet, these curves can also be produced when high doses cause opposing effects to low doses, such as *the down regulation* of cell proliferation, which is biologically distinct from cell death [61, 62]. Importantly, even though NMDRCs can manifest in apical endpoints due to the interaction of two monotonic curves at lower levels of biological organization, they cannot be generalized based on this feature because the apical endpoints and underlying mechanisms vary significantly. Other mechanisms responsible for NMDRCs have been described in detail including the expression of cell- and tissue-specific receptors and cofactors, receptor selectivity and competing interactions of multiple receptors, receptor downregulation and desensitization, receptor competition, endocrine negative feedback loops, and others [2].

Principle 5: The effects of hormones are life-stage dependent

When hormone exposures occur in adulthood, they are referred to as “activational” because the body responds by being ‘activated’, and when exposure ceases, the effects also cease [63]. An example of this is the effect of relatively high estrogen doses on adult female fertility; estrogen exposures prevent ovulation in women and female animals, but when administration of estrogens stops, ovulation is re-initiated [64]. In contrast to these activational effects, exposures during early development are termed “organizational” because they can completely ‘re-organize’ target tissues in the developing embryo/fetus/neonate [65]. For example, estrogen has a role during fetal development in organizing the sexually dimorphic regions of the brain [66]. Further, abnormal estrogen exposures cause

structural malformations of the male reproductive tract [67] and increase the susceptibility of the female mammary gland to adult cancers [68–70].

2.1.1 How weight-of-evidence (WoE) approaches can incorporate these endocrine principles

WoE analyses are defined as a “type of consideration made in a situation where there is uncertainty, and which is used to ascertain whether the evidence or information supporting one side of a cause or argument is greater than that supporting the other side” [71]. As defined, for the purposes of chemical safety evaluations, WoE analyses allow for the balancing of studies that show effects of a chemical in question with those studies that show no effect. Importantly, they do not require consensus among all studies. It has also been noted that WoE evaluations involve scientific and extra-scientific values, which are often not clear, obvious, or even acknowledged [72]. This may explain why 30% of toxicologists surveyed for their opinions on the evaluation of chemical risks concluded that risk assessment is not an objective scientific process [73]. Finally, WoE analyses always involve professional judgment [74]; the problem with this is that risk assessors and other scientists can examine the same data and come to very different conclusions based on the role of judgment [73, 75–77]. The lack of reproducibility of WoE analyses – as well as other forms of risk assessment – is rarely discussed in the fields of toxicology and risk assessment, but it must be acknowledged [78].

There is general consensus that WoE approaches should analyze all data available as opposed to many risk assessments that begin by selecting “the best” or “most relevant” studies based on “professional judgment” (i.e. usually studies that meet arbitrary criteria such as a sample size of ‘n’ animals/group rather than those that use power analysis to identify the fewest number of animals necessary) [79]. Some WoE analyses have focused on the effects of a chemical on a single endpoint [80], whereas others have examined the effects of chemical exposures more generally, on a wider range of biological processes [81]. However, the use of WoE analyses to scrutinize studies of EDCs is relatively new, and no standardized approaches have been developed for this class of chemicals [82]. Even though several WoE analyses have been conducted on chemicals with known endocrine activities (for example [83–85]), these analyses have failed to incorporate the basic principles of endocrinology.

In more detail, WoE analyses are expected to include all data, but most weigh some studies based on *a priori* criteria as better than other studies. For example, Harvey and Johnson write, “Where available for chemicals, [Good Laboratory Practice (GLP)] studies are taken to provide the strongest evidence of endocrine effects, *or their absence*, and should accordingly be weighted above less comprehensive data” [86]. This statement is interesting for two reasons: first, it makes clear that specific parameters, i.e. GLP guidelines, which generally dictate data collection procedures and not data quality [87, 88], can be considered to change the ‘value’ of a study. GLP regulations were instituted in response to misconduct in private research companies, and were designed to ensure that basic guidelines are met (i.e. appropriate training of research staff, calibration of equipment, collection and storage of raw data). The employment of GLP guidelines is not evidence for appropriate study design or study quality [43, 88–91]. Second, this statement suggests some scientists believe the *absence of evidence of harm* should be considered *evidence of no harm*. Studies that are designed to test the effects of a chemical use the scientific process of posing the null hypothesis that “Chemical X produces no differences from the negative control.” Statistics are used to refute the null hypothesis and conclude that “Chemical X does have effects that are distinct from the negative control” with a specific probability of error. The inability to refute the null hypothesis (that is, failure to achieve a particular level of statistical

significance) *cannot be used* to conclude that “Chemical X is the equivalent to the negative control”; failure to refute the null hypothesis is not equivalent to proving it is correct [92].

In our WoE analysis, we identified several criteria that we used to ‘value’ or ‘weight’ different studies [2]. Unlike the criteria that are heavily valued by Harvey and Johnson [86] and many risk assessors (i.e. use of GLP), the criteria we selected are founded in the principles of endocrinology. For example, we established *a priori* that studies must demonstrate that 1) there is no evidence of contamination by the test chemical, natural hormones, or other hormone mimics/blockers in the negative controls (or any other treatment group); 2) the experimental system is capable of responding to low doses; and 3) sensitive species and strains were used. Importantly, in our analysis, these criteria were applied equally to studies that do and do not show effects of the chemical. However, the conclusions that could be made from the inclusion of positive controls are quite different when studies show an effect of a chemical versus when they do not (see [90, 93] and Figure 1). Rhomberg and Goodman [41] and others [94] have challenged the requirement for appropriate positive and negative controls in EDC studies. However, the use of these controls is not only a central tenet of endocrinology, these criteria are also widely accepted – and expected for appropriate experimental design – in many fields of experimental biology [95].

2.2 Issue #2: Is there consensus on whether “low dose effects” exist?

More than a decade ago, the NTP expert panel concluded that low dose effects “have been demonstrated in laboratory animals exposed to certain endocrine-active agents” [4]. This conclusion was drawn from a relatively small number of studies; at that time, in-depth analyses were limited by the number of chemicals that had been studied at doses below the established toxicological NOAEL. Yet, from the NTP analysis alone, it is clear that a variety of experts from various scientific disciplines believed the evidence was strong enough to conclude that there were low dose effects for at least a subset of EDCs.

Does the NTP’s 2002 publication represent a field-wide consensus on this issue? Surely it should not be claimed that all scientists agree about the presence of low dose effects; few fields in science can truly claim that consensus (e.g. widespread agreement, unanimity) exists. What is clear is that the NTP panel – comprised of scientists from academia, government and industry – did reach a level of agreement on the existence of low dose effects for a small number of EDCs.

One of the chemicals the NTP did not reach a decisive conclusion about was bisphenol A (BPA), a chemical found in a large number of consumer products and environmental sources including food and beverage can linings, polycarbonate plastics, and thermal papers, among others [96, 97]. The NTP panel found that “[s]everal studies provide credible evidence for low-dose effects of bisphenol A” including effects on prostate weight, timing of female puberty, and strain-specific effects on serum prolactin levels, but their analyses were confounded by other studies that found no evidence of low dose effects for the same endpoints [4]. Importantly, the NTP *hinted at underlying endocrine principles* when they wrote: “For those studies that included DES exposure groups [a positive control for estrogenicity], those that showed an effect with bisphenol A showed a similar low-dose effect with DES (e.g., prostate and uterus enlargement in mice); those that showed no effect with bisphenol A also found no effect with DES.” Further, the NTP panel noted that some studies that showed no effects of BPA were hindered by the use of an insensitive strain of rat. Subsequent analyses show that a large number of studies concluding no effect of BPA have utilized this strain [90, 98]. While Rhomberg and Goodman argue for the use of insensitive strains by suggesting that sensitive strains are “so prone to developing a

particular effect that [their] responses to chemicals overestimate human risks” [41], this notion directly contradicts the NTP expert panel’s conclusion that “animal-model selection should be based on responsiveness to endocrine-active agents of concern (i.e., responsive to positive controls)” [4].

In our recent review [2], we chose the example of BPA and the prostate and determined whether the studies with the highest value based on *a priori* established endocrine principles would provide better agreement on the effects of BPA on development of the prostate. We found substantial evidence for low dose effects on prostate weight, and additional supportive evidence that BPA exposure predisposes the prostate to hormone-induced prostatic intraepithelial neoplasia (PIN) lesions [2]. Several of the more recent studies were conducted in the laboratories of scientists that had served on the NTP’s 2002 low dose expert panel, which was unable to make conclusive remarks about BPA’s low dose activities at that time. Our conclusion – that there are low dose effects of BPA on the prostate – is consistent with the NTP’s 2008 decision on BPA, which found that there was *some concern* for the effects of BPA on the prostate gland of fetuses, infants and children at current human exposure levels [99, 100]. This scientific evaluation and conclusion was endorsed by the US FDA in 2010 as well [101].

In a second WoE analysis of BPA, we evaluated the literature examining the effects of BPA on the development of the mammary gland. In a striking example of consistency, almost every low dose study that we could identify (exposures < 400 µg/kg/day), collected from multiple independent laboratories, showed effects on the mammary gland including increased sensitivity to chemical carcinogens [2]; the two studies that showed no effects of BPA were difficult to interpret due to their study design and lack of information specific to the mammary gland. Importantly, there were effects of BPA reported even at doses below the US EPA reference dose of 50 µg/kg/day [102], providing some evidence that the current regulatory practice, which uses safety factors to calculate a “safe” intake dose, is not sufficient. After the publication of our review, similar results to those reported in the mouse and rat were described in a non-human primate model [103], where developmental exposures to doses of BPA far below the NOAEL were shown to alter parameters of mammary gland organization. Together with the voluminous and remarkably consistent rodent data, this study provides strong evidence in support of the hypothesis that BPA could alter development of the human breast at levels of exposure below that which is generally regarded as safe by the regulatory process.

Although BPA has been extremely well studied, it is not likely to be a model for all EDCs. For example, a recent ToxCast screen performed by the EPA indicated that BPA binds to a large number of hormone receptors and was given the 3rd highest score (of the 309 chemicals studied) in a WoE toxicological priority index [104]. Its effects, therefore, are expected to be more variable than other EDCs with narrower targets. In our original review, we also performed a WoE analysis on the effects of atrazine on male sexual differentiation in amphibians [2], another highly controversial issue [105]. We and others believe the implications for low dose effects on wildlife cannot be ignored [106, 107], even when comparisons between route of exposure and dosing methods are difficult to relate to humans [108]. In fact, ‘low dose effects’ are relevant to the health of wildlife populations [106, 109, 110], and thus need not be directly pertinent to human health to deserve significant attention. When using the principles of endocrinology in our atrazine WoE analysis, it became very clear that atrazine affects male sexual development in amphibians at low doses [2]. Taking into account all studies examining the effects of atrazine on amphibian sexual development, we concluded that there is sufficient evidence to reject the null hypothesis that low doses of atrazine are safe; on the contrary, there is sufficient evidence that atrazine exerts adverse effects on male amphibians at doses below the accepted safe dose.

In total, we focused our analysis of the low dose paradigm on five examples where a relatively large number of studies were designed to examine the effects of a specific EDC on a particular endpoint. We also, however, collected other examples of low dose effects for approximately 20 additional chemicals. When we use the NTP's standards (of at least three studies to analyze), there remain a significant number of chemicals with confirmed low dose effects (Table 2). Although we did not perform WoE analyses for each of these additional examples, they collectively provide convincing evidence that low dose effects are observable across different classes of chemicals and for EDCs with differing mechanisms of action. They also provide support to indicate that low dose effects occur in a variety of species and animal strains, consistent with the high level of conservation of the endocrine system across the animal kingdom [111–115].

We have proposed that it is important to evaluate the 'low dose paradigm' as it has been studied in controlled laboratory settings within the context of results produced in the field of epidemiology. Although this is a topic that is deserving of its own detailed review, an increasing number of studies link EDC exposures to human health issues (reviewed in [116–119]). Furthermore, it was these epidemiology studies that provided the impetus to re-visit the low dose paradigm (see for example [120–126]).

Rhomberg and Goodman [41] suggested that the examples we highlighted in our review are “anecdotes” that we present “without any attempt to review all of the available pertinent data” (see Table 1). Not only is this not true – and Rhomberg and Goodman do not present any examples of low dose papers we failed to include – this argument also ignores the fact that studies focusing on low doses remain relatively uncommon; our reliance on a small number of studies (relative to the large number of toxicological studies) is not by choice, but rather because the vast majority of EDCs have not been studied in the low dose range, regardless of how “low dose” is defined [2]. This observation clearly demonstrates that chemicals to which the human population is exposed are not tested at doses that are calculated to be safe, but are deemed to be safe based on assumptions that are not germane to chemicals that interfere with hormone action.

2.3 Issue # 3: Debate surrounding non-monotonicity in EDC studies

If EDCs can produce adverse effects on biological systems at doses below the toxicologically-derived “safe” level, then how are EDCs different from general toxins such that this occurs? The shape of the dose-response of hormones and EDCs acting on hormone action is one of the important underlying reasons. For several years, basic scientists and risk assessors have debated the presence and relevance of NMDRCs for chemicals including EDCs [127]. Unlike the term ‘low dose’, which is a relative term, non-monotonicity is defined mathematically as a response where the slope of the dose response curve changes sign. In principle, non-monotonicity can occur anywhere in the dose range and thus can occur at low doses, at high doses, or over a range including both high and low doses. In our review, we examined several hundred examples of NMDRCs, identified for more than 20 natural hormones and more than 70 EDCs including DEHP, octylphenol, BPA, nonylphenol, genistein, resveratrol, quercetin, dioxin, PCBs, atrazine, endosulfan, dieldrin, DDT, prochloraz, DES, PBDEs, triclocarban, methoxychlor, chlorpyrifos, vinclozolin, PFOA, and selenium, among many others [2]. These NMDRCs were observed in cultured cells, whole animals, and in human populations for a variety of endpoints (see Table 3). Rhomberg and Goodman and others have challenged our analysis by claiming that 1) we have not proven that any particular case is reproducible, 2) we have not determined the actual frequency of NMDRCs for EDCs, 3) there is no evidence for NMDRCs in guideline studies, 4) NMDRCs are observed in some tissues but not others, 5) there is no evidence that NMDRCs occur in the range of human exposures, 6) a mechanism has not been shown for

each example of non-monotonicity, 7) there can be high temporal variability for human exposures to environmental chemicals and therefore NMDRCs are statistical ‘flukes’, and 8) we have not shown whether any of these NMDRCs – or any of the low dose effects – are adverse. We address each of these issues below, with the final point addressed in the last section of this review.

2.3.1 Are NMDRCs reproducible?

NMDRCs are a common feature in endocrinology and pharmacology studies, and in clinical situations are sometimes referred to as ‘flare’ [128, 129]. The question of whether they are reproducible depends on the availability of multiple studies that have examined the same or similar treatment under the same or similar conditions. This has clearly been done in clinical cases of tamoxifen, a pharmaceutical estrogen receptor antagonist, which produces NMDRCs: when first administered to women, low concentrations in blood stimulate tumor growth, and then as the internal concentrations increase, high doses inhibit tumor growth [130–133]. It has also been demonstrated in clinical cases of Lupron treatment, a pharmaceutical agonist of gonadotropin releasing hormone, which also produces NMDRCs: at low doses, Lupron stimulates FSH and therefore induces ovulation, whereas high doses suppress the ovarian response [134, 135].

Similarly, in experimental science, several studies have reported NMDRCs for mammary growth parameters in mice exposed to estradiol and DES [136–139]. NMDRCs have been reported for the effects of estrogens on cell proliferation and a number of endpoints associated with membrane estrogen receptor signaling, and the effects of androgens on prostate cell number (reviewed in [28, 61, 140–143]).

2.3.2 What is the frequency of NMDRCs?

If NMDRCs are a very rare event, then current regulatory practices, which extrapolate expected effects at low doses from the effects observed at high doses, may not be confounded by their presence. But if NMDRCs are common then they may be an important contributor to the failure of the current regulatory approach to accurately determine the risk of exposure to EDCs. In our review, we did not undertake the formidable task of determining the frequency of NMDRCs in the EDC literature. Yet others have performed estimates using various sampling techniques of the general toxicology literature, and calculate that NMDRCs (and in particular U- or inverted U-shaped curves) occur in 12–24% of all dose response studies [144]. Those authors note that the studies most likely to detect NMDRCs include at least six doses, and that their reported frequency of NMDRCs could underestimate its incidence due to bias against publishing studies that show non-monotonicity; this bias exists in toxicology because the absence of a monotonic dose-response curve is typically interpreted as an absence of a dose-related response.

Other estimates of NMDRC frequencies have focused on hormetic responses, a specific kind of NMDRC that is defined by a range of doses that stimulate effects over the response seen in the control and other doses that inhibit effects below what is seen in the control [145]; these curves are often interpreted as showing that a chemical has ‘beneficial’ effects at low doses and ‘detrimental’ or toxic effects at high doses [146]. Although few EDC studies meet the criteria for hormesis and it is generally accepted that hormesis is not applicable to EDC studies [147–150], it is worth noting that several studies estimate that hormetic curves occur in more than 20% of toxicology studies, including a large number that detect significant effects below the NOAEL [151–153]. Although these estimates are not directly relevant to the question of how frequent NMDRCs are in EDC studies, they do challenge the use of high dose to low dose extrapolation in current regulatory practices.

Are NMDRCs expected for all chemicals? There is no support for this hypothesis; instead, the hypothesis best supported by data is that NMDRCs are expected for chemicals and pharmaceuticals that interact with receptors. Because of their interference with hormone receptor signaling, EDCs are an important class of chemicals that are expected to produce NMDRCs.

2.3.3 Why aren't NMDRCs reported in guideline studies?

Guideline studies are internationally agreed-upon testing methods, typically used in industry and government laboratories, to characterize the hazards of chemicals and chemical mixtures. These studies typically examine what have been called 'apical' endpoints, i.e. endpoints at the highest level of biological organization. For example, guideline reproductive toxicity assays are expected to examine body weight and food consumption in the dam, timing of gestation, number of pups and uterine implantation sites, sex ratios, weight of organs, and histopathology for reproductive organs [154]. Guideline studies have been challenged for their lack of sensitivity for endocrine endpoints [43, 88], yet these remain the studies that risk assessors value highest [94, 155]. The question of whether guideline studies can detect NMDRCs is therefore an important one based largely on regulators' reliance on these studies for chemical safety decisions. Importantly, NMDRCs have been detected in guideline studies (for example [156–159]), although they are often dismissed as “paradoxical” or irrelevant, or even explained due to errors in study conduct [160]. For example, a recent study examining the effects of a flame retardant mixture identified NMDRCs in two-generation and developmental toxicity studies for endpoints such as litter size and congenital defects [161]; the authors note that NMDRCs were dismissed in these guideline studies as “spurious” or “unrelated to dose”.

There is also a question of whether NMDRCs are observed in categorical endpoints (i.e. tumor/no tumor, death/no death) or whether they are specific to continuous endpoints (i.e. body weight, age at menopause, etc.) To address this question, we examined bioassays for possible carcinogenesis available in the NTP's public collection (OECD guideline studies for carcinogenesis). We found NMDRCs for survival rates in male mice exposed to chlordane (an insecticide), the incidence of liver neoplasias in response to heptachlor (an insecticide), and the incidence of thyroid adenoma and pituitary adenoma in female rats, as well as the incidence of liver carcinoma and incidence of animals with malignant tumors in male mice exposed to lindane (an insecticide) [162]. Clearly, these NMDRCs are present in various kinds of guideline studies, even for all-or-none endpoints, if studies are examined carefully.

2.3.4 Are NMDRCs relevant if they are only observed in some tissues?

This question originates from the assumption in regulatory toxicity testing for EDCs that one endpoint of hormone action is a proxy measure of all endpoints of that hormone. However, this assumption is clearly false based on two principle observations. First, endogenous hormone signaling is cell-, tissue- and organ-specific. The specificity of hormone action can be accounted for by having different hormone receptors mediate hormone action on different endpoints, as well as by other cell-specific mechanism. Second, it is clear that EDCs that act as imperfect ligands on hormone receptors will not faithfully replicate the effects of the native hormone.

One would never dismiss the specificity of a carcinogen for its ability to induce tumors in one tissue but not another as irrelevant to health, because we have a reasonable understanding of this specificity in some cases. Therefore, a better question might be whether some tissues and organs are more prone to exhibit NMDRCs than others; this issue requires additional study.

2.3.5 Do NMDRCs occur in the range of human exposures?

Rhomberg and Goodman suggest that there is no evidence for NMDRCs in the range of human exposures [41]. This assertion ignores the large number of NMDRCs we reported in epidemiology studies for a range of chemicals and endpoints (Table 3). Clearly, these studies, which overwhelmingly focus on environmentally exposed adults, indicate that NMDRCs are occurring in the range of actual human exposures – at doses that are generally considered ‘safe’.

Unfortunately, our knowledge of actual human exposure levels – as well as exposure routes and sources – is quite limited for the majority of EDCs. This means that studying ‘human relevant doses’ in animals can be quite difficult. Although we have some information about the range of blood or urine levels for some of these chemicals due to biomonitoring programs such as the CDC’s NHANES (see for example [163–167]), for the majority of EDCs, we know very little about what applied concentration is required to produce those internal measures.

Finally, it is worth asking whether NMDRCs *must occur* in the range of human exposures *to be relevant for risk assessment*. Clearly, if a non-monotonic relationship occurs between the doses tested in traditional toxicology studies (i.e. the NOAEL or NOEL) and the calculated “safe” or reference dose, this would still have serious implications for risk assessment (see [168] for a practical example – this example demonstrates how assumptions of linearity influence risk assessment decision-making). We have focused on NMDRCs because their existence indicates that high dose testing, combined with extrapolations (often using a series of 10-fold safety factors) to calculate a ‘safe’ dose, is confounded by their presence. Thus, the question of whether NMDRCs occur in the range of human exposures is not necessarily germane to addressing whether high to low dose extrapolations are appropriate.

2.3.6 Do we know the mechanism responsible for each example of non-monotonicity?

In our recent review and above, we discussed several mechanisms known to produce NMDRCs [2]. Most of these mechanisms were characterized in extensive detail *in vitro*, both in cell culture and cell-free experiments following analysis of natural hormones. They are well-acknowledged in the field of endocrinology. Studies have also explored the mechanisms responsible for NMDRCs in tissues, organs, whole animals and even populations; some of these mechanisms are the same as those that have been clearly demonstrated *in vitro*, but it is also likely that the mechanisms operating *in vivo* are more complicated than those characterized in cultured cells.

Although Rhomberg & Goodman [41] recognize these mechanisms, they write that our review did not show “that these mechanisms actually operate for particular EDCs.” It is indeed a curious requirement that a study show exactly which mechanism is operating in order for an observation to be accepted as ‘real’, as has occurred in the study of EDCs. Whether or not we know *exactly which mechanism* is responsible for each non-monotonic effect has no bearing on whether these observations exist. The public health literature has many examples where a mechanism is not immediately understood to explain an important phenomenon. For example, in the 1850’s, John Snow traced the source of a London cholera outbreak to the public water supply, but had no ‘mechanistic’ explanation for it – this lack of understanding of germ theory at that time (i.e. the absence of a mechanism) did not change the fact that an infectious agent was responsible, and that the city’s water pump should be disassembled for the purpose of protecting public health [169]. Thus, regardless of whether we can explain the mechanistic underpinnings of each NMDRC, and at what level of biological complexity they are best understood, their existence alone challenges traditional means of risk assessment.

2.3.7 Can we distinguish NMDRCs reported in epidemiology studies from statistical 'flukes' of sampling?

Rhomberg & Goodman [41] argue that the NMDRCs observed in human populations could be due to various kinds of sampling errors including temporal fluctuations in blood/urine concentrations of EDCs, which have been reported for chemicals such as BPA that are metabolized within hours of exposure [170]. Interpreting studies depends on use of appropriate statistics and interpretation of statistical results. Thus, it is important that analyses of NMDRCs distinguish between random biological fluctuations in results and true examples of non-monotonicity. Rhomberg & Goodman's argument fails to consider that most of the chemicals that produce NMDRCs in epidemiological studies are persistent organic pollutants (POPs) – chemicals that bioaccumulate and therefore produce consistent exposure measurements in human samples over long periods of time. Further, in contrast to the claim that EDC exposures normally encompass small ranges, for many of the POPs wide ranges are observed, allowing for better separation between low-exposure and high-exposure groups (see for example [171]).

2.4 Issue #4: Distinguishing adverse effects from adaptive effects

In the discussions surrounding our review, one criticism has been whether the endpoints that have been examined represent adverse effects. This is important because the NOAEL marks the highest dose where no *adverse* effects have been observed in traditional toxicology studies; it does not mark the dose where no effects *at all* were observed. The question of whether the low dose effects and NMDRCs we have reported represent adverse effects hinges on the definition of adverse. The US EPA defines an adverse effect as “a biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge” [172]. The IPCS/OECD defines an adverse effect as “a change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences” [173]. Neither FDA nor EFSA have a definition for adverse effect, and decisions on adversity are left solely to the expert judgment of risk assessors. Unfortunately, for chemicals found in food, this leaves a wide degree of latitude for chemicals to impact human health. For example, if a food contaminant causes a person to get a headache or diarrhea, the FDA would not consider this adverse [127], even though many food contaminants are consumed regularly and therefore affected individuals could be ill daily. Also important, many guideline studies do not test for the full complement of ‘adverse’ effects – i.e. there is no examination of whether a test chemical alters an animal's ability to “compensate for additional stress” or alters the “susceptibility to... other environmental influences” later in life.

The absence of agreed upon definitions for adversity leaves the field in a significant conundrum, and therefore, it is not valid to assert that endpoints identified in low dose studies are not adverse if the regulatory community has yet to agree on a set of criteria that should be employed in the definition of adverse. Importantly, most of the endpoints we identified that are significantly affected by low doses and/or display NMDRCs are considered *deleterious by endocrinologists and/or developmental biologists, and many are likely to be considered adverse* by at least some risk assessors (Table 2, Table 3). Further, the endpoints observed in humans exposed to environmentally relevant levels of EDCs are all typically considered to be deleterious health conditions including diabetes, obesity, high fasting glucose levels, abnormally high cholesterol levels, abnormal bone mineral density, presence of atherosclerotic plaques, arthritis, endometriosis and low mental development scores.

In the toxicology community, adverse effects are often defined by the timing of their manifestation relative to the exposure period. Based on the traditional definition of an ‘adverse effect’, chemical exposures that occur *in utero* are expected to produce malformations or defects that are apparent at birth. Examples include cleft palate, spina bifida, hypospadias, microcephaly, and dextrocardia. Yet many EDCs produce obvious adverse effects – infertility, cancers, etc. – that do not manifest for years or even decades following exposure. The sad example of DES showed how exposures in the womb would have drastic, adverse health consequences that would not be realized in human patients until they reached puberty, reproductive maturity and even menopause [174–177].

The assessment of an endpoint as adverse or not often depends on context and the available scientific information. For example, decreased anogenital distance in male rodents is indicative of fetal exposure to chemicals that block testosterone action, with additional associated adverse effects [178]. Yet, examining the same measure in human infants – and showing an inverse correlation with prenatal phthalate exposure [121, 179] – was challenged as both non-adverse and lacking biological plausibility [180]. Scientific exploration has shown associations between shortened anogenital distance in infant boys and the incidence of hypospadias [181]; additional associations have been observed between shortened anogenital distance in men and poor semen quality [182], lower serum testosterone levels [183], and decreased fertility [184]. It has also been suggested that shortened anogenital distance could be a biomarker of testicular dysgenesis syndrome [185]. Thus, while the first human study was immediately contested as “not adverse”, this negation was based on a lack of information rather than based on fact.

It is also important to note the population-based consequences of small, seemingly non-adverse effects of chemical exposure. For many years it was thought that the small decrease in IQ associated with developmental lead exposure was not adverse because there are no ‘real’ consequences for the average person losing 5 IQ points [186, 187]. However, a population-based analysis shows what happens when a normally distributed feature, like IQ, gets shifted even slightly: the number of children classified as mentally retarded increases significantly [188]. It is now acknowledged that there is no threshold below which lead is considered safe for children [187]. There are countless other examples of normally distributed continuous variables such as hypertension, adult height, penile length, and serum hormone levels where even slight shifts (~5%) could have serious physical, mental, financial or social consequences for a portion of the population [189]. Thus, ‘non-adverse’ changes on an individual level can have serious, detrimental effects on the population, which should not be ignored.

Finally, it has been suggested that, rather than produce adverse effects, the types of responses we and others have reported following EDC exposures should be considered ‘adaptive’ [41, 190]. Hormones are often thought of as the ‘thermostat’ of the bodily functions, and the concept of ‘adaptive’ effects is an extension of that metaphor; shifts in the ‘thermostatic controls’ are expected to be accommodated and adjusted by the organism. To some degree, that may be true in adults. As discussed earlier in this review, adults are affected by hormones (and by extension EDCs) during the period of exposure, but the effects are activational and therefore they often, but not always, recede when exposures end [191, 192]. Exceptions to this dogma include a recent study that shows that adult female mice exposed to low levels of BPA only during pregnancy had altered metabolic responses months after the short period of exposure ceased [193]. Additional exceptions include EDC exposures that are continuous or EDCs that bioaccumulate, thus the effects may be activational, but the response is constantly ‘activated’ because the chemical is always present and therefore having an effect.

The bigger danger in the ‘adaptive’ model for EDCs, however, is that it completely disregards the role of hormones in the developing individual [194]. Individuals with deficits in thyroid hormone during fetal development are born with mental retardation [50]. Males exposed to estrogens or anti-androgens in the womb can develop severe congenital defects of the reproductive tract [195]. What is ‘adaptive’ about these consequences?

The plasticity – or adaptability – of the developing embryo allows it to survive some serious challenges during development, but not without consequences that are strongly related to the postnatal environment. A large dataset showing adaptive responses in humans examined the link between fetal malnutrition and a host of adult diseases including heart disease, hypertension, type 2 diabetes and stroke [196–198]. This phenomenon was termed the “thrifty phenotype” because the data suggested that poor nutrition during early development led to an increased susceptibility to nutritional problems *as a result of a more affluent diet later in life* [199]. This adaptive response is best described with a metaphor [200]: “The poorly nourished mother essentially gives the fetus a forecast of the nutritional environment into which it will be born. Processes are set in motion which lead to a postnatal metabolism adapted to survival under conditions of poor nutrition. The adaptations only become detrimental when the postnatal environment differs from the mother’s forecast, with an over abundance of nutrients and consequent obesity.” Thus, the adaptive response of the individual (i.e. increased blood flow to the brain of the malnourished fetus at the expense of the visceral organs) allows it to live – but it will be successful (i.e. healthy) only in a very particular environment. When that environment does not exist, however, the individual is detrimentally affected.

A similar example demonstrates the role of natural hormones in producing offspring with variable phenotypes; female rodents positioned between two female siblings in the uterus are more docile as adults and are more likely to attract mates when resources are plentiful [201–204]. However, when resources are scarce, females that were positioned between two male siblings in the uterus, who are more aggressive than other females, are more likely to mate. Thus, an EDC exposure that mimics or blocks the sex hormones involved in establishing these behaviors during fetal development could cause all females to have one phenotype or the other. Whether or not they are reproductively successful will then depend on whether resources are plentiful or scarce. Importantly, these responses to low doses of hormones during gestation are not limited to rodents; small changes in intrauterine hormonal exposures associated with twin pregnancies affect important reproductive endpoints, as well as other physiological processes, in humans as well (see for example [70, 205–210]).

Together, these examples illustrate that an argument that EDC exposures produce “adaptive” rather than “adverse” effects requires knowledge about environmental conditions that are not available, nor are they capable of being controlled by the exposed individual. To have a logical discussion about this very complex issue, it is imperative that those who favor the interpretation of EDC effects as reflective of adaptive responses support that interpretation with genuine scientific data rather than what amounts to wishful thinking that utilizes an outdated and scientifically inaccurate concept of hormone action across the lifespan.

3. Conclusions

The discussion of low dose effects and NMDRCs is a scientific one, and has been analyzed using knowledge from a large number of scientific disciplines. However, what we have learned from basic science has yet to influence how chemical safety evaluations are performed. We and others have discussed at length how the principles of endocrinology are relevant, and must be the foundation for our understanding of how EDCs behave. We have yet to see any scientific argument that successfully rebuts this stance. We have also

presented voluminous amounts of data showing that 1) EDCs have effects on laboratory animals, wildlife and humans at doses that are considered safe by traditional toxicology testing, and 2) that many EDCs produce NMDRCs, which offers one challenge to the assumption that ‘safe’ doses can be extrapolated from high dose testing. We recognize that these data challenge the traditional ways of regulating chemicals. However, we propose the consideration of two questions to provide focus and clarity: Has the case for low dose effects and NMDRCs been sufficiently refuted? And, are the default assumptions of monotonicity and endpoint continuity valid?

Rhomberg and Goodman state that if our interpretation of the low dose and NMDRC data is correct, “there would be profound consequences for toxicity testing and its interpretation in risk analysis and safety assessment” [41]. We understand that these findings challenge risk assessment dogma, but society’s tendency to maintain the *status quo* is insufficient as an argument to rebut scientific data. The many observations of chemical exposures being associated with human disease cannot be dismissed as irrelevant *in toto*, especially when there is so much evidence within the field of endocrinology to support the interpretation that low doses exert adverse effects on the human population. Data must trump theories, hypotheses, models and assumptions, and not the reverse.

Our 2012 review has been criticized for not having a detailed solution to these fundamental problems. Although we have proposed specific changes to current chemical safety assessments (i.e. testing of doses in the range of human exposures, expansion of endpoints included in testing protocols, the incorporation of endocrine principles in the assessment of EDCs, the use of power analysis to determine sample sizes, the proper utilization of epidemiological data, etc.), we recognize that identifying the problem is usually easier than fixing it. In her 2012 editorial [38], NIEHS director Dr. Linda Birnbaum stated: “It is time to start the conversation between environmental health scientists, toxicologists, and risk assessors to determine how our understanding of low-dose effects and nonmonotonic dose responses influence the way risk assessments are performed for chemicals with endocrine-disrupting activities.” Once the data demonstrating low dose effects and NMDRCs are acknowledged by scientists across disciplines, we will be able to work together toward effective solutions.

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Abbreviations

EDC	endocrine disrupting chemical
DES	diethylstilbestrol
GLP	good laboratory practices
NHANES	National Health and Nutrition Examination Survey
NMDRC	non-monotonic dose response curve
NOAEL	no observed adverse effect level
NOEL	no observed effect level

POP	persistent organic pollutant
WoE	weight of evidence

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Figure 1.

A decision tree utilized for WoE analyses, incorporating principles of endocrinology. This decision tree illustrates how high value studies were selected for the five WoE examples conducted previously [2].

Table 1

Misleading or incorrect statements from the abstract of Rhomberg & Goodman, with responses

Statement or Implication in Abstract	Actual Fact
"... [Vandenberg et al.] present examples as anecdotes ..."	Examples are clearly referenced, which is why the reference list contains more than 840 citations. In addition, specific data from examples are shown in tables within the paper; this is the opposite of anecdotal presentation.
"... without attempting to review all available pertinent data, ..."	For each example analyzed in detail, we presented every reference available in the peer-reviewed scientific literature. In the text, Rhomberg & Goodman do not point out any specific studies we failed to include.
"...selectively citing studies without evaluating most of them ..."	For our analyses of low dose effects, we selected five examples to discuss in detail. Here, all studies – whether they supported the hypothesis or refuted it – were included in the analysis; selective citation did not occur. For our analyses of NMDRCs, tabulation of the results was performed specifically to evaluate the breadth of this literature. However, we used expert judgment to evaluate which studies were the major ones to receive detailed attention. We did not exclude any studies that disagree with our perspective that NMDRCs are common; on the contrary, there are no scientific studies rebutting the presence of NMDRCs in the EDC literature or their frequency.
"... examining whether their putative examples ..."	The actual findings and data from papers were compiled in tables; the numerical data are not "putative" but are the published results of the studies.
"... are consistent and coherent with other relevant information. ..."	Consistent and coherent evaluation of the literature was the purpose of the entire review, and the process and standards used in evaluation were detailed in the paper (Section II, Parts A & B, pages 389 to 394), to make the consistency and coherence of the process explicit; the criteria for evaluation were specifically detailed in Sections II.B.1–3.
"... assume that any statistically significant association indicates causation ..."	Controlled experimental design IS the scientific standard for the demonstration of causation, and for experimental science (i.e. bench science) statistical significance IS the scientific standard for evaluating experimental effects. Of course, statistical significance must always be evaluated as a means of excluding random chance.
"... studies with positive results are evaluated differently than those with null results ..."	As described in the original review, and in further detail in this paper, using Endocrine Principles, the controls and scientific standards for statistical significance ARE different for positive and negative findings: positive findings require a valid negative control, while negative findings require a valid positive control.
"... They also do not evaluate whether exposures in studies are truly 'low-dose' and relevant to humans ..."	Table 1 in the paper compiles and addresses the several definitions of low-dose explicitly, with tabulation of all of the low-doses for the two examples of BPA and DEHP by four standard definitions of low-dose; two of those four definitions (first and fourth) are of human exposure. The human relevant activity levels for all of the major endocrine disrupting chemical hormone groups are tabulated in Table 2; the numbers used as the cut-offs for "low-dose" for over 45 chemicals are detailed in Tables 3 and 4; and the numbers used for the cut-offs for "low-dose" studies for about 28 chemicals in animal and human studies are detailed in Table 3. Evaluation of human relevant low-doses was one of the major points of the paper.
"They propose a number of different nonmonotonic dose–response curves, but do not consider reasons for why they should be expected to apply generally across species."	Explicitly addressed in the paper are the widely published conservation of hormone action mechanisms across species, and of chemicals with hormonal activity. The mechanisms of NMDRCs and how they apply across species were detailed in the individual sections on each specific mechanism (pages 406 through 409); the citations of work across species documents that they apply across species, and is cited in the references of each section.
"Many of their examples would be – and indeed have been – questioned by many scientists ..."	All studies should be questioned by scientists – this is the nature of the scientific process. However, what this statement seems to refer to is the "questioning" of studies on the premise of generating doubt – for the sake of doubt. Studies should be evaluated for quality; in the case of EDCs, the scientific quality of many industry-funded studies is very low by endocrine standards. The Endocrine Society has recently released an evaluation of the risk assessment of endocrine disruptors which is relevant to this question [44].
"... while overlooking evidence that suggests the contrary."	Our paper explicitly evaluated and compared positive and negative studies by weight of evidence standards which were detailed in the review.

Table 2

An updated low dose effect table using n=3 studies as cut-off

Chemical	Use	EDC action	Low dose cut-off	Affected endpoint	References
Aroclor 1221 (PCB mixture)	Coolants, lubricants, paints, plastics	Mimic estrogens, anti-estrogenic activity, etc.	0.1–1 mg/kg (produces human blood levels)	Brain sexual dimorphisms	[6–8]
Atrazine	Herbicide	Induces aromatase expression	200 µg/L	Male sexual differentiation/development (amphibians)	11 studies reviewed in [2]
Bisphenol A	Plastics, epoxy resins, thermal papers	Binds ER, mER, ERRγ, PPARγ. May weakly bind AR, TR	5–50mg/kg (LOAEL, US EPA) Conservative cut-off: 400µg/kg/day	Prostate weight, sensitivity of adult prostate to hormones, mammary gland development, sensitivity of mammary gland to carcinogens	12 prostate studies and 17 mammary gland studies reviewed in [2]
Chlorpyrifos	Insecticide	Anti-androgenic	1mg/kg/day (US EPA)	Acetylcholine receptor activity (brain)	[211–213]
DDT	Insecticide	Binds ER	0.05mg/kg (US EPA)	Neurobehavior	[9–15]
Diethylstilbestrol (DES)	Pharmaceutical	Binds ER	0.02 µg/kg/day	Prostate weight	Identified by the NTP expert panel [4]
Dioxin (TCDD)	Industrial byproduct	Binds AhR	1 µg/kg/day	Spermatogenesis	17 studies reviewed in [2]
Estradiol	Natural estrogen	Binds AhR	3 µg/kg/day	Serum prolactin, serum LH, serum FSH	Identified by the NTP expert panel [4]
Genistein	Isoflavone derived from soy	Binds ER	25ppm in food	Sexual dimorphisms of the brain, male mammary gland development, proliferation of T-lymphocytes	Identified by the NTP expert panel [4]
Methoxychlor	Insecticide	Binds ER	10ppm in food	Immune system effects	Identified by the NTP expert panel [4]
Nicotine	Natural alkaloid in tobacco	Binds acetylcholine receptors, stimulates epinephrine	Human use of nicotine substitutes	Incidence of male genital defects, i.e. cryptorchidism, hypospadias (Humans)	[16–19]
Nonylphenol	Industrial compound, surfactant	Binds ER	25ppm in food	Brain sexual dimorphisms, relative thymus weight, proliferation of T-lymphocytes, prolonged estrus	Identified by the NTP expert panel [4]
Octylphenol	Rubber bonding, surfactant	Weakly binds ER, RXR, PRGR	10mg/kg/day [214]	Testes endpoints	[20–23]
Perchlorate	Fuel, fireworks	Blocks iodide uptake, alters thyroid hormone	0.4 mg/kg/day	TSH levels (humans)	4 studies, reviewed in [2]
Tributyltin oxide	Pesticide, wood preservation	Binds PPARγ	0.19mg/kg/day (US EPA)	Obesity	[24–27]

Table 3

endpoints assessed in NMDRC studies

Examples of non-monotonic endpoints identified in cell culture experiments			
Cell number	Dopamine uptake	Prolactin release	Phosphorylation of target proteins
Cytokine release	Calcium flux	Mitochondrial oxidation	Testosterone release
Number of bacterial colonies	Retinoic acid activity	Estrogen levels	Gene expression
Aromatase activity	Corticosterone levels	Receptor activation	
Examples of non-monotonic endpoints identified in animal experiments			
Mammary gland morphology	Reproductive organ weight*	Effects on anxiety behaviors*	Response to neurodegenerative drugs*
Locomotor activity*	Gene expression	Bone growth	Insulin production*
Sexual behaviors*	Embryo number*	Fertility*	Timing of vaginal opening*
Aromatase activity in hypothalamus	Serum cholesterol levels*	Body weight*	Responses to allergens*
Fecundity	Corticosterone levels*	Testosterone levels*	Cell-mediated immunity
Memory*	Aggressive behaviors*	Time to metamorphosis*	Estrous cyclicity*
Sex ratios*	Male anogenital distance*	Protein expression	Survivorship patterns*
Examples of non-monotonic endpoints identified in epidemiology studies			
Incidence of coronary events*	Depression*	Mortality*	Risk of hip fractures*
Incidence of Alzheimer's disease*	BMI*	Waist circumference	Diabetes incidence*
Telomere length in lymphocytes	Triglyceride levels*	Cholesterol levels*	Risk of rapid infant weight gain*
Bone mineral density*	Atherosclerotic plaques*	Arthritis*	Endometriosis*
Sperm count*	Mental development scores (infants)*	Hypertension*	Incidence of metabolic syndrome*

All of these endpoints would be considered deleterious by endocrinologists, developmental biologists and/or primary care physicians.

We have marked (*) the endpoints that we expect at least some risk assessors would consider adverse.