

Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses

Laura N. Vandenberg, Theo Colborn, Tyrone B. Hayes, Jerrold J. Heindel, David R. Jacobs, Jr., Duk-Hee Lee, Toshi Shioda, Ana M. Soto, Frederick S. vom Saal, Wade V. Welshons, R. Thomas Zoeller, and John Peterson Myers

Center for Regenerative and Developmental Biology and Department of Biology (L.N.V.), Tufts University, Medford, Massachusetts 02155; The Endocrine Disruption Exchange (T.C.), Paonia, Colorado 81428; Laboratory for Integrative Studies in Amphibian Biology (T.B.H.), Molecular Toxicology, Group in Endocrinology, Energy and Resources Group, Museum of Vertebrate Zoology, and Department of Integrative Biology, University of California, Berkeley, California 94720; Division of Extramural Research and Training (J.J.H.), National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, Research Triangle Park, North Carolina 27709; Division of Epidemiology and Community Health (D.R.J.), School of Public Health, University of Minnesota, Minneapolis, Minnesota 55455; Department of Preventive Medicine (D.-H.L.), School of Medicine, Kyungpook National University, Daegu 702-701, Korea; Molecular Profiling Laboratory (T.S.), Massachusetts General Hospital Center for Cancer Research, Charlestown, Massachusetts 02129; Department of Anatomy and Cellular Biology (A.M.S.), Tufts University School of Medicine, Boston, Massachusetts 02111; Division of Biological Sciences (F.S.v.S.) and Department of Biomedical Sciences (W.V.W.), University of Missouri-Columbia, Columbia, Missouri 65211; Biology Department (T.Z.), University of Massachusetts-Amherst, Amherst, Massachusetts 01003; and Environmental Health Sciences (J.P.M.), Charlottesville, Virginia 22902

For decades, studies of endocrine-disrupting chemicals (EDCs) have challenged traditional concepts in toxicology, in particular the dogma of “the dose makes the poison,” because EDCs can have effects at low doses that are not predicted by effects at higher doses. Here, we review two major concepts in EDC studies: low dose and nonmonotonicity. Low-dose effects were defined by the National Toxicology Program as those that occur in the range of human exposures or effects observed at doses below those used for traditional toxicological studies. We review the mechanistic data for low-dose effects and use a weight-of-evidence approach to analyze five examples from the EDC literature. Additionally, we explore nonmonotonic dose-response curves, defined as a nonlinear relationship between dose and effect where the slope of the curve changes sign somewhere within the range of doses examined. We provide a detailed discussion of the mechanisms responsible for generating these phenomena, plus hundreds of examples from the cell culture, animal, and epidemiology literature. We illustrate that nonmonotonic responses and low-dose effects are remarkably common in studies of natural hormones and EDCs. Whether low doses of EDCs influence certain human disorders is no longer conjecture, because epidemiological studies show that environmental exposures to EDCs are associated with human diseases and disabilities. We conclude that when nonmonotonic dose-response curves occur, the effects of low doses cannot be predicted by the effects observed at high doses. Thus, fundamental changes in chemical testing and safety determination are needed to protect human health. (*Endocrine Reviews* 33: 0000–0000, 2012)

I. Introduction

- A. Background: low-dose exposure
- B. Background: NMDRCs
- C. Low-dose studies: a decade after the NTP panel’s assessment
- D. Why examine low-dose studies now?
- E. Mechanisms for low-dose effects
- F. Intrauterine position and human twins: examples of natural low-dose effects

II. Demonstrating Low-Dose Effects Using a WoE Approach

- A. Use of a WoE approach in low-dose EDC studies
- B. Refuting low-dose studies: criteria required for acceptance of studies that find no effect
- C. BPA and the prostate: contested effects at low doses?
- D. BPA and the mammary gland: undisputed evidence for low-dose effects

Abbreviations: A4, Androstenedione; AhR, aryl hydrocarbon receptor; BPA, bisphenol A; CDC, Centers for Disease Control and Prevention; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DES, diethylstilbestrol; EDC, endocrine-disrupting chemicals; EPA, Environmental Protection Agency; ER, estrogen receptor; FDA, Food and Drug Administration; GLP, good laboratory practices; LOAEL, lowest observed adverse effect level; mER, membrane-associated ER; NHANES, National Health and Nutrition Examination Survey; NIS, sodium/iodide symporter; NMDRC, nonmonotonic dose-response curve; NOEL, no observed effect level; NOAEL, no observed adverse effect level; NTP, National Toxicology Program; PIN, prostatic intraepithelial neoplasias; POP, persistent organic pollutants; ppb, parts per billion; SERM, selective ER modulator; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; WoE, weight of evidence.

- E. Another controversial low-dose example: atrazine and amphibian sexual development
- F. Dioxin and spermatogenesis: low-dose effects from the most potent endocrine disruptor?
- G. Perchlorate and thyroid: low-dose effects in humans?
- H. Low-dose summary
- III. Nonmonotonicity in EDC Studies
 - A. Why is nonmonotonicity important?
 - B. Mechanisms for NMDRCs
 - C. Examples of nonmonotonicity
 - D. NMDRC summary
- IV. Implications of Low-Dose Effects and Nonmonotonicity
 - A. Experimental design
 - B. Regulatory science
 - C. Human health
 - D. Wildlife
- V. Summary

I. Introduction

This review focuses on two major issues in the study of endocrine-disrupting chemicals (EDCs): low-dose exposures and nonmonotonic dose-response curves (NMDRCs). These concepts are interrelated, and NMDRCs are especially problematic for assessing potential impacts of exposure when nonmonotonicity is evident at levels of exposure below those that are typically used in toxicological assessments. For clarity of presentation, however, we will first examine each of the concepts separately.

A. Background: low-dose exposure

It is well established in the endocrine literature that natural hormones act at extremely low serum concentrations, typically in the picomolar to nanomolar range. Many studies published in the peer-reviewed literature document that EDCs can act in the nanomolar to micromolar range, and some show activity at picomolar levels.

1. What is meant by low dose?

In 2001, at the request of the U.S. Environmental Protection Agency (EPA), the National Toxicology Program

(NTP) assembled a group of scientists to perform a review of the low-dose EDC literature (1). At that time, the NTP panel defined low-dose effects as any biological changes 1) occurring in the range of typical human exposures or 2) occurring at doses lower than those typically used in standard testing protocols, *i.e.* doses below those tested in traditional toxicology assessments (2). Other definitions of low dose include 3) a dose below the lowest dose at which a biological change (or damage) for a specific chemical has been measured in the past, *i.e.* any dose below the lowest observed effect level or lowest observed adverse effect level (LOAEL) (3), or 4) a dose administered to an animal that produces blood concentrations of that chemical in the range of what has been measured in the general human population (*i.e.* not exposed occupationally, and often referred to as an environmentally relevant dose because it creates an internal dose relevant to concentrations of the chemical measured in humans) (4, 5). This last definition takes into account differences in chemical metabolism and pharmacokinetics (*i.e.* absorption, distribution, and excretion of the chemical) across species and reduces the importance of route of exposure by directly comparing similar blood or other tissue concentrations across model systems and experimental paradigms. Although these different definitions may seem quite similar, using just a single well-studied chemical like bisphenol A (BPA) shows how these definitions produce different cutoffs for exposure concentrations that are considered low dose (Table 1). For many chemicals, including EDCs, a large number of studies meet the criteria for low-dose studies regardless of whether the cutoff point for a low dose was based on the range of typical human exposures, doses used in traditional toxicology, or doses that use an internal measure of body burden.

Whether low doses of EDCs influence disease is a question that now extends beyond the laboratory bench, because epidemiological studies show that environmental exposures to these chemicals are associated with disorders in humans as well (see for examples Refs. 6–16). Although disease associations have historically been observed in individuals exposed to large concentrations of EDCs after

TABLE 1. Low-dose definitions and cutoff doses: BPA and DEHP as examples

Chemical	Estimated range of human exposures	Doses below the NOAEL	Doses below the LOAEL	Administered doses (to animals) that produce blood levels in typical humans
BPA	0.4–5 $\mu\text{g}/\text{kg} \cdot \text{d}$ (679)	No NOAEL was ever established in toxicological studies (38)	<50 $\text{mg}/\text{kg} \cdot \text{d}$ (38)	~400 $\mu\text{g}/\text{kg} \cdot \text{d}$ to rodents and nonhuman primates (4, 253)
DEHP	0.5–25 $\mu\text{g}/\text{kg} \cdot \text{d}$ (680)	<5.8 $\text{mg}/\text{kg} \cdot \text{d}$ (681, 682)	<29 $\text{mg}/\text{kg} \cdot \text{d}$ (681, 682)	Unknown

Estimates of human exposure are made from consumer product consumption data but do not take into account that there are unknown sources of these chemicals. DEHP, Bis(2-ethylhexyl) phthalate.

industrial accidents (17–19) or via occupational applications (20–22), recent epidemiological studies reveal links between environmentally relevant low concentrations and disease prevalence. With the extensive biomonitoring studies performed by the U.S. Centers for Disease Control and Prevention (CDC) (23, 24) and similar environmental surveys performed in Europe (25) and elsewhere (www.statcan.gc.ca/concepts/hs-es/measures-mesures-eng.htm), knowledge about environmental exposures to EDCs and their associations with human health disorders has increased substantially.

Low-dose effects have received considerable attention from the scientific and regulatory communities, especially when examined for single well-studied chemicals like BPA (4, 27–32). The low-dose literature as a whole, however, has not been carefully examined for more than a decade. Furthermore, this body of literature has been disregarded or considered insignificant by many (33, 34). Since the NTP's review of the low-dose literature in 2001 (2), a very large body of data has been published including 1) additional striking examples of low-dose effects from exposures to well-characterized EDCs as well as other chemicals, 2) an understanding of the mechanisms responsible for these low-dose effects, 3) exploration of nonmonotonicity in *in vivo* and *in vitro* systems, and 4) epidemiological support for both low-dose effects and NMDRCs.

2. Is the term low dose a misnomer?

Endogenous hormones are active at extremely low doses, within and below the picomolar range for endogenous estrogens and estrogenic drugs, whereas environmental estrogen mimics are typically active in the nanomolar to micromolar range (for examples, see Refs. 35–38), although some show effects at even lower concentrations (39–41). Importantly, the definitions above do not take into account the potency or efficacy of the chemical in question, a topic that will be discussed in greater detail below. Instead, low dose provides an operational definition, in which doses that are in the range of human exposure, or doses below those traditionally tested in toxicological studies, are considered low. To be clear, none of these definitions suggest that a single concentration can be set as a low dose cutoff for all chemicals. Using the above definitions, for some chemicals, low doses could potentially be in the nanogram per kilogram range, but for most chemicals, doses in the traditional micro- and milligram per kilogram range could be considered low doses because traditional approaches to testing chemicals typically did not examine doses below the milligram per kilogram dose range.

B. Background: NMDRCs

We have defined low-dose studies according to the definitions established by the NTP panel of experts (2). However, because the types of endpoints that are typically examined at high doses in toxicological studies are often different from the types of endpoints examined in low-dose studies, one cannot assume that an effect reported in the low-dose range is necessarily different from what would be observed at higher doses. For example, low doses of a chemical could affect expression of a hormone receptor in the hypothalamus, an endpoint not examined in high-dose toxicology testing, and high doses could similarly affect this same endpoint (but are likely to be unreported because high doses are rarely tested for these types of endpoints). Thus, the presence of low-dose effects makes no assumptions about what has been observed at higher concentrations. (As discussed elsewhere, for the majority of chemicals in commerce, there are no data on health effects and thus no established high- or low-dose range.) Therefore, low-dose effects could be observed at the lower end of a monotonic or linear dose-response curve.

In contrast, the definition of a NMDRC is based upon the mathematical definition of nonmonotonicity: that the slope of the dose-response curve changes sign from positive to negative or vice versa at some point along the range of doses examined (42). Often NMDRCs have a U- or inverted U-shape (43); these NMDRCs are thus also often referred to as biphasic dose-response curves because responses show ascending and descending phases in relation to dose. Complex, multiphasic curves have also been observed (41, 44, 45). NMDRCs need not span from true low doses to high (pharmacologically relevant) doses, although experiments with such a broad dose range have been performed for several EDCs; the observation of nonmonotonicity makes no assumptions about the range of doses tested. Examples of NMDRCs from *in vitro* cell culture and *in vivo* animal experiments, as well as epidemiological examples, are presented in detail later in this review (see *Sections III.C.1–3*). Additional examples of NMDRCs are available in studies examining the effects of vitamins and other essential elements on various endpoints (see for example (46)); these will not be examined in detail in this review due to space constraints.

NMDRCs present an important challenge to traditional approaches in regulatory toxicology, which assume that the dose-response curve is monotonic. For all monotonic responses, the observed effects may be linear or nonlinear, but the slope does not change sign. This assumption justifies using high-dose testing as the standard for assessing chemical safety. When it is violated, high-dose testing regimes cannot be used to assess the safety of low doses.

It should be noted that both low dose and nonmonotonicity are distinguished from the concept of hormesis, which is defined as a specific type of response whereby “the various points along [the dose response] curve can be interpreted as beneficial or detrimental, depending on the biological or ecological context in which they occur” (47). Estimations of beneficial or adverse effects cannot be ascertained from the direction of the slope of a dose-response curve (48–50). In their 2001 Low Dose Peer Review, the NTP expert panel declined to consider whether any effect was adverse because “in many cases, the long-term health consequences of altered endocrine function during development have not been fully characterized” (2). There are still debates over how to define adverse effects (51–53), so for the purposes of this review, we consider any biological change to be an effect. Importantly, most epidemiological studies are by definition examining low doses (unless they are focusing on occupationally exposed individuals), and these studies typically focus on endpoints that are accepted to be adverse for human health, although some important exceptions exist (54–56).

Finally, it is worth noting that any biological effect, whether it is observed to follow linear relationships with administered dose or not, provides conclusive evidence that an EDC has biological activity. Thus, other biological effects are likely to be present but may remain undetected or unexamined. Many EDCs, including those used as pesticides, were designed to have biological effects (for example, insecticides designed to mimic molting hormone). Thus, the question of whether these chemicals have biological effects is answered unequivocally in their design; the question is what other effects are induced by these biologically active agents, not whether they exist.

C. Low-dose studies: a decade after the NTP panel's assessment

In 2000, the EPA requested that the NTP assemble a panel of experts to evaluate the scientific evidence for low-dose effects and dose-response relationships in the field of endocrine disruption. The EPA proposed that an independent and open peer review of the available evidence would allow for a sound foundation on which the EPA could “determine what aspects, if any, of its standard guidelines for reproductive and developmental toxicity testing [would] need to be modified to detect and characterize low-dose effects” (2). The NTP panel verified that low-dose effects were observed for a multitude of endpoints for specific EDCs including diethylstilbestrol (DES), genistein, methoxychlor, and nonylphenol. The panel identified uncertainties around low-dose effects after exposure to BPA; although BPA had low-dose effects on some endpoints in some laboratories, others were not

found to be consistent, leading the panel to conclude that it was “not persuaded that a low-dose effect of BPA has been conclusively established as a general or reproducible finding” (2).

Since the NTP's review of low-dose endocrine disruptor studies, only a few published analyses have reexamined the low-dose hypothesis from a broad perspective. In 2002, R. J. Witorsch (57) analyzed low doses of xenoestrogens and their relevance to human health, considering the different physiologies associated with pregnancy in the mouse and human. He proposed that low doses of endocrine disruptors would not likely affect humans because, although low-dose effects had been observed in rodents, the hormonal milieu, organs controlling hormonal release, and blood levels of estrogen achieved are quite different in humans. There are, of course, differences in hormones and hormone targets between rodents and humans (58), but the view that these differences negate all knowledge gained from animal studies is not supported by evolutionary theory (59–61). This human-centered stance argues against the use of animals for any regulatory testing (62) and runs counter to the similarities in effects of EDCs on humans and animals; rodents proved to be highly predictive of the effects of DES on humans (63, 64). In a striking example, studies from mice and rats predicted that gestational exposure to DES would increase mammary cancer incidence decades before women exposed *in utero* reached the age where this increase in risk was actually observed (65–67).

In 2007, M. A. Kamrin (68) examined the low-dose literature, focusing on BPA as a test case. He suggested that three criteria were required to support the low-dose hypothesis. First is reproducibility, which he defined as “the same results are seen from the same causes each time a study is conducted.” Furthermore, he proposed that the dose response for the effects must be the same from study to study. Second is consistency, which he defined as the results all fitting into a pattern, whereby the results collected from multiple species and under variable conditions all show the same effect. And third is proper conduct of studies, which he defined as including the appropriate controls and performance under suitable experimental conditions as well as the inclusion of multiple doses such that a dose-response curve can be obtained.

Although we and others (69–72) agree with the use of these criteria (reproducibility, consistency, and proper experimental design), there are significant weaknesses in the logic Kamrin employed to define these factors. First, suggesting that reproducibility is equivalent to the same results obtained each time a study is conducted is unrealistic and not a true representation of what is required of replication. As has been discussed in other fields, “there is no

end to the ways in which any two experiments can be counted as the same — or different . . . All experiments are the same in respect of their being experiments; they are all different by virtue of being done at different places, at different times, by different people, with different strains of rat, training regime, and so on” (73).

Furthermore, according to the Bradford-Hill criteria, a set of requirements accepted in the field of epidemiology to provide adequate evidence of a causal relationship between two factors, a single negative result (or even several studies showing negative results) cannot negate other studies that show adverse effects (74). Essentially, all scientists know that it is very easy for an experiment to find no significant effects due to a myriad of reasons; it is more difficult to actually find effects, particularly when using highly sophisticated techniques (69).

Second, the concept of consistency as a pattern that can be derived from all results is one we will use below, using a weight-of-evidence (WoE) approach and several specific examples. However, Kamrin’s proposed idea that every study must show the same effect has the same weaknesses as discussed for the proposed definition of reproducibility and does not acknowledge the obvious differences in many species and strains. It also suggests that the identification of a single insensitive strain could negate any number of positive studies conducted with appropriate animal models (75).

And finally, Kamrin suggested that only studies with appropriate controls should be used for analyses, a criterion we agree should be followed. However, his own scrutiny of the low-dose animal literature fails to do so (68). He also suggested that studies use multiple doses so that a dose-response curve can be obtained. Although studies using a single dose can be informative, we agree that dose-response relationships provide important information to researchers and risk assessors alike. However, this requirement is not helpful if there is an insistence on observing a linear response; as we discuss in depth in this review, there are hundreds of examples of nonmonotonic and other nonlinear relationships between dose and endpoint. These should not be ignored.

In 2004, Hayes (76) reviewed the available literature concerning the effects of atrazine on amphibian development, with a specific focus on the effect of ecologically relevant doses of this EDC on malformations of the gonads and other sexually dimorphic structures; in the case of aquatic exposures, it can be difficult to determine what a cutoff for a low dose would be; thus, Hayes focused on studies examining the effects of atrazine at levels that had been measured in the environment. He reviewed the results produced by several labs, in which it was independently demonstrated that low concentrations of atrazine

produced gonadal abnormalities including hermaphroditism, males with extra testes, discontinuous gonads, and other defects. Hayes’ work also clearly addressed the so-called irreproducibility of these findings by analyzing the studies that were unable to find effects of the pesticide; he noted that the negative studies had multiple experimental flaws, including contamination of the controls with atrazine, overcrowding (and therefore underdosing) of experimental animals, and other problems with animal husbandry that led to mortality rates above 80%.

In 2006, vom Saal and Welshons (77) examined the low-dose BPA literature, identifying more than 100 studies published as of July 2005 that reported significant effects of BPA below the established LOAEL, of which 40 studies reported adverse effects below the 50 $\mu\text{g}/\text{kg} \cdot \text{d}$ safe dose set by the EPA and U.S. Food and Drug Administration (FDA); all of these studies would be considered low dose according to the NTP’s definition (2). The authors proposed that these examples should be used as evidence to support the low-dose hypothesis. Furthermore, this publication detailed the similarities among the studies that were unable to detect any effects of low doses of BPA and established a set of criteria required to accept negative studies. We have adapted the criteria detailed by Hayes (76) and vom Saal and Welshons (77) to produce a set of requirements for low-dose studies; these criteria are described in some detail below.

D. Why examine low-dose studies now?

The developmental origins of health and disease hypothesis originated from studies showing that fetal DES exposure could cause severe malformations and cancers of the reproductive tract, and other studies demonstrating that fetal malnutrition could lead to adult diseases including metabolic syndrome, diabetes, and increased stroke incidence (78–81). Since that time, the developmental origins of health and disease hypothesis has been extended to address whether diseases that are increasing in prevalence in human populations could be caused by developmental exposures to EDCs (67, 82–85). Evidence from the animal literature has been tremendously informative about the effects of EDC exposures early in development and has driven new hypotheses to be tested in epidemiology studies (86). Studies including several discussed in this review provide supportive evidence that the fetal and neonatal periods are specifically sensitive to chemicals that alter endocrine signaling and that EDCs could be contributing to a range of diseases.

Strong, reliable, and reproducible evidence documents the presence of low concentrations of EDCs and other chemicals in human tissues and fluids, as well as in environmental samples (28, 87–89). These studies indicate

that samples collected from humans and the environment typically contain hundreds of contaminants, usually in the parts-per-billion (ppb) range (90, 91). The obvious question with potentially large public health implications is whether these concentrations are so low as to be irrelevant to human health. The fact that epidemiological analyses (reviewed in *Section III.C.3*) repeatedly find associations between the measured concentrations in human samples and disease endpoints suggests it is inappropriate to assume the exposures are too low to matter. That is especially the case given the empirical data (reviewed in *Section II.A*) from animal and cell culture experiments showing effects can be caused by concentrations comparable (and sometimes below) what is measured in humans and also the detection of NMDRCs in some of those same experiments.

In the human biomonitoring field, large databases such as the CDC's National Health and Nutrition Examination Survey (NHANES) have allowed researchers to make comparisons between groups of individuals with various exposure criteria; some of these studies will be addressed in detail in subsequent sections of this review. Although by definition these databases examine low-dose exposures, their use has been the subject of significant debate. Because of the large number of chemicals that have been measured (>300 in the most recent NHANES by the CDC) and the large number of health outcomes and other disease-related data collected from the individuals that donated biological samples, it has been argued that the number of possible associations that could be made would lead to a significant number of false positives (92); thus, associations could be found simply because of extensive data dredging. This has led some to suggest that these studies as a whole should be rejected (93, 94).

In response to these criticisms, epidemiologist Jan Vandenberg (95) notes, "researchers do not mindlessly grind out one analysis after another"; the examination of these databases for associations between chemical exposures and health effects does not entail the statistical comparison between all possible factors, calculated as some 8800 comparisons in the CDC's NHANES database (92). Instead, epidemiologists typically focus on a select number of comparisons that address relationships between chemicals and diseases identified *a priori* (96, 97), often because of mechanistic data obtained in laboratory animals or *in vitro* work with human and animal cells and tissues. Repeated findings of links between EDC exposures and diseases in epidemiological analyses of biomonitoring data based on *a priori* hypotheses suggests these relationships should not be rejected as a statistical artifact and, instead, should be the basis for significant concern that low-dose effects can be detected in the general population (85, 98).

E. Mechanisms for low-dose effects

The endocrine system is particularly tuned to respond to very low concentrations of hormone, which allows an enormous number of hormonally active molecules to coexist in circulation (38). As a ligand-receptor system, hormones act by binding to receptors in the cell membrane, cytosol, or the nucleus. The classical effects of nuclear hormone receptors influence gene expression directly, although rapid nongenomic actions at membrane-associated receptors are now well documented and accepted. Membrane receptors are linked to different proteins in the cell, and binding to these receptors typically changes cellular responses in a rapid fashion (99), although the consequence of a rapid signaling event could be the activation of a nuclear transcription factor, leading to responses that take longer to detect. Peptide hormones can also influence gene expression directly (see Refs. 100 and 101 for examples).

There are several means by which the endocrine system displays specificity of responses to natural hormones. Many hormone receptors are expressed specifically in a single or a few cell types (for example, receptors for TSH are localized to the thyroid), whereas some (like thyroid hormone receptors) are found throughout the body (102). For receptors that are found in multiple cell types, different effects are produced in part due to the presence of different coregulators that influence behaviors of the target genes (103–105). And finally, some hormones have multiple receptors [for example estrogen receptor (ER) α and ER β], which are expressed in different quantities in different cell types and organs and can produce variable effects on gene expression or cellular phenomena (cell proliferation *vs.* apoptosis) (102, 106).

The typical physiological levels of the endogenous hormones are extremely low, in the range of 10–900 pg/ml for estradiol, 300–10,000 pg/ml for testosterone, and 8–27 pg/ml for T₄ (see Table 2). Importantly, steroid hormones in the blood are distributed into three phases: free, representing the unconjugated, unbound form; bioavailable, representing hormones bound to low-affinity carrier proteins such as albumin; and inactive, representing the form that is bound to high-affinity binding proteins such as SHBG or α -fetoprotein (38) (Fig. 1A). When the circulating levels in blood are corrected for the low fraction of the hormones that are not bound to serum binding proteins, the free concentrations that actually bring about effects in cells are even lower, for example 0.1–9 pg/ml for estradiol. Concentrations of active hormones will vary based on the age and physiological status of the individual (*i.e.* plasma testosterone levels are less than 1 ng/ml in male children but increase to approximately 5–7 ng/ml in adulthood; during menses, estradiol levels are typically less than 100

TABLE 2. Ranges of endogenous hormones in humans (from Ref. 108)

Hormone	Free concentration (females)	Total concentration (females)	Free concentration (males)	Total concentration (males)
Cortisol	20–300 ng/ml		20–300 ng/ml	
Estradiol	0.5–9 pg/ml (adult female)	<20 pg/ml (prepubertal) 20–800 pg/ml (premenopausal) <30 pg/ml (postmenopausal)		10–60 pg/ml (adult)
Progesterone		0.2–0.55 ng/ml (prepubertal) 0.02–0.80 ng/ml (follicular phase) 0.90–4 ng/ml (luteal phase) <0.5 ng/ml (postmenopausal)		0.1–0.4 ng/ml (prepubertal) 0.2–2 ng/ml (adult)
Insulin		0–250 pmol/liter		0–250 pmol/liter
GH		2–6 ng/ml		2–6 ng/ml
Prolactin		0–15 ng/ml		0–10 ng/ml
Testosterone	9–150 pg/ml (adult)		0.3–250 ng/ml	
Thyroid hormone	8–30 pg/ml (10–35 pM)		8–30 pg/ml (10–35 pM)	
TSH	0.5–5 μ U/ml		0.5–5 μ U/ml	

pg/ml, but just before ovulation, they spike to 800 pg/ml; *etc.*) (107, 108). Of course, it should be noted that active concentrations of natural hormones vary somewhat from species to species and can even vary between strains of the same species (109).

There are several reasons why endogenous hormones are able to act at such low circulating concentrations: 1) the receptors specific for the hormone have such high affinity that they can bind sufficient molecules of the hormone to trigger a response, 2) there is a nonlinear relationship between hormone concentration and the number of bound receptors, and 3) there is also a nonlinear relationship between the number of bound receptors and the strongest observable biological effect. Welshons and colleagues (38) describe how hormone concentration influences receptor occupancy: “receptor occupancy is never determined to be linear in relation to hormone concentration . . . At concentrations above the K_d [the dissociation constant for receptor-ligand binding kinetics], saturation of the response occurs first, and then at higher concentrations, saturation of receptors is observed.” What this means is that at low doses of hormone, a 10-fold increase in hormone concentration can have a 9-fold increase in receptor occupancy, whereas at high doses of hormone, a 10-fold increase in hormone concentration produces a less than 1.1-fold increase in receptor occupancy (38) (Fig. 1B). Thus, even moderate changes in hormone concentration in the low-dose range can produce substantial changes in receptor occupancy and therefore generate significant changes in biological effects. Welshons *et al.* (38) also note that a near-maximum biological response can be observed without a high rate of receptor occupancy, a situation that was previously termed the spare receptor hypothesis (110, 111); that is, the response mechanism saturates before all of the receptors are saturated.

The presence of spare receptors is the basis for saying that these receptor systems are tuned to detect low concentrations that lead to occupancy of 0.1–10% of total receptors. Within this range of low receptor occupancy, there is high proportionality between changes in the free hormone concentration and changes in receptor occupancy, and a change in receptor occupancy by a ligand for the receptor is required to initiate changes in receptor-mediated responses (38).

There are additional reasons why natural hormones are active at low doses: 4) hormones have a strong affinity for their receptors (relative to affinity for other receptors) because many hormones are secreted from a single gland or site in the body but must have effects throughout the body in multiple tissues and 5) blood concentrations of hormones are normally pulsatile in nature, with the release of one hormone often controlled by the pulsatile release of another hormone (112, 113), and both the frequency and the amplitude of pulses modulate the biological response; hormones are also influenced by circadian rhythms, with dramatic differences in hormone secretion depending on the time of day (114, 115).

For many years, the mechanisms by which some environmental chemicals acted at low doses were not well understood. In 1995, the National Research Council appointed the Committee on Hormonally Active Agents in the Environment to address public concerns about the potential for adverse effects of EDCs on human health (116). At the time, work on understanding the mechanisms by which EDCs exert their effects was in its infancy, and in the executive summary, the committee stated, “Lack of knowledge about a mechanism does not mean that a reported effect is unconfirmed or unimportant, nor does demonstration of a mechanism document that the resulting effects are unique to that mechanism or are pervasive

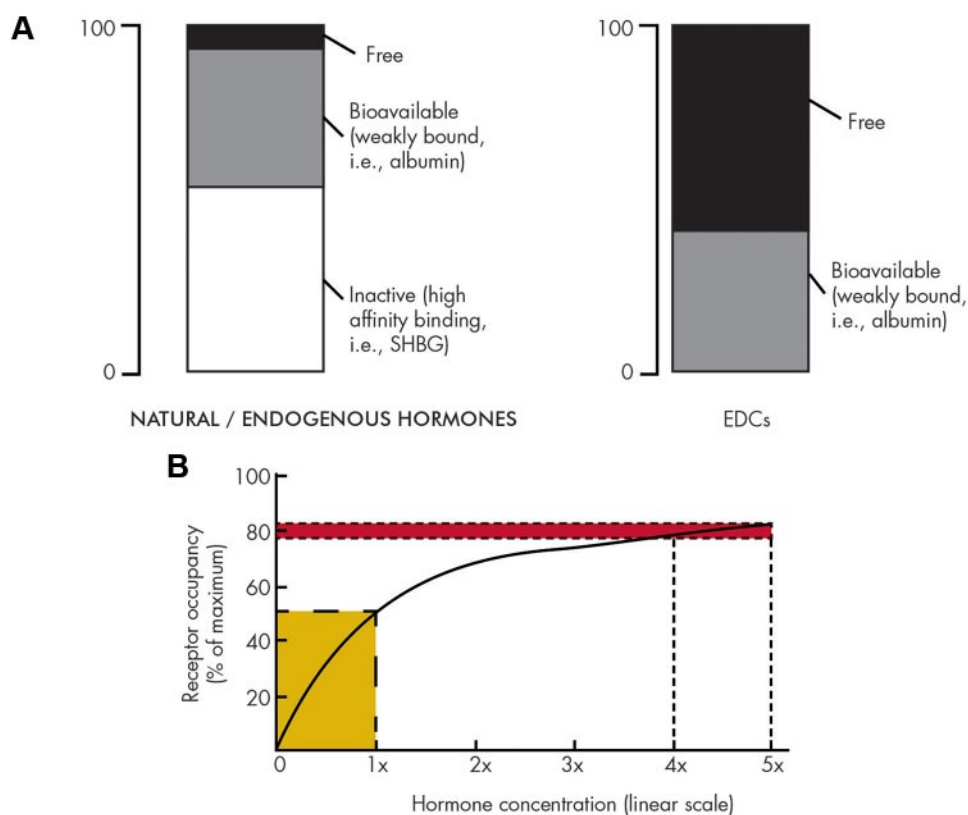
Figure 1.

Figure 1. Characteristics and activities of natural hormones. A, This schematic depicts a typical relationship of three phases of circulating hormones: free (the active form of the hormone), bioavailable (bound weakly to proteins such as albumin), and inactive (bound with high affinity to proteins such as SHBG). These three phases act as a buffering system, allowing hormone to be accessible in the blood, but preventing large doses of physiologically active hormone from circulating. With EDCs, there may be little or no portion maintained in the inactive phase. Thus, the entirety or majority of a circulating EDC can be physiologically active; the natural buffering system is not present, and even a low concentration of an EDC can disrupt the natural balance of endogenous hormones in circulation. B, Schematic example of the relationship between receptor occupancy and hormone concentration. In this theoretical example, at low concentrations, an increase in hormone concentration of x (from 0 to $1x$) causes an increase in receptor occupancy of approximately 50% (from 0 to 50%, see *yellow box*.) Yet the same increase in hormone concentration at higher doses (from $4x$ to $5x$) causes an increase in receptor occupancy of only approximately 4% (from 78 to 82%, see *red box*).

in natural systems.” Since that time, a tremendous amount of work has been dedicated to understanding the molecular mechanisms of action of EDCs, and in particular the mechanisms responsible for low-dose effects.

1. General mechanisms for EDC action

As discussed above, the endocrine system evolved to function when unbound physiologically active ligands (hormones) are present at extremely low doses (117). Because of shared receptor-mediated mechanisms, EDCs that mimic natural hormones have been proposed to follow the same rules and therefore have biological effects at low doses (38, 118). Similarly, EDCs that influence in any way the production, metabolism, uptake, or release of hormones also have effects at low doses, because even small changes in hormone concentration can have biologically important consequences (38, 119).

The estrogen-response mechanisms have been extensively studied with regard to the effects of endogenous estrogens and estrogenic drugs. In classical, genomic estrogen action, when endogenous estrogens bind to ER, those receptors bind to estrogen response element sequences or to a number of other response element sites adjacent to the genes directly responsive to estrogens; this binding influences transcription of estrogen-sensitive genes (120). Xenoestrogens produce the same reactions; these chemicals bind to ERs, which then initiate a cascade of molecular effects that ultimately modify gene expression. Therefore, for the actions of estrogenic EDCs, molecular mechanisms and targets are already known in some detail. Similar mechanisms are induced by the binding of androgens to the androgen receptor, or thyroid hormone agonists to the thyroid hormone receptor, among others.

Additionally, there are EDCs that act as antagonists of these hormone systems, binding to a receptor, but not activating the receptor's typical response, and preventing the binding or activity of the endogenous ligand. Finally, many EDCs bind to the receptor and trigger a response that is not necessarily the same as that triggered by the endogenous estrogens; these are termed selective ER modulators (SERMs). Ultimately, all of these actions occur at the level of the receptor.

Many studies have been dedicated to the understanding of which EDCs bind to which nuclear hormone receptors and how the binding affinities compare to the natural steroid. Thus, many of these chemicals have been classified as weak hormones. Yet studies have shown that, for example, the so-called weak estrogens like BPA can be equally potent as endogenous hormones in some systems, causing biological effects at picomolar levels (30, 38, 41, 121). Both endogenous estrogens and EDCs can bind to ER associated with the cell membrane [membrane-associated ER (mER) α and mER β] that are identical to the nuclear ER (122–124), and a transmembrane ER called G-protein coupled receptor 30 that is structurally dissimilar to the nuclear ER and encoded by a distinct gene (125, 126). In many cells, 5–10% of total ER α and ER β are localized to the plasma membrane (124); these membrane-associated receptors are capable of nongenomic steroid action in various cell types (30, 121, 127); thus, rapid and potent effects are well documented for many EDCs including BPA, DES, endosulfan, dichlorodiphenyldichloroethylene (DDE), dieldrin, and nonylphenol, among others (41, 128–130).

Finally, EDCs have other effects that are not dependent on binding to either classical or membrane-bound steroid hormone receptors. EDCs can influence the metabolism of natural hormones, thus producing differences in the amount of hormone that is available for binding either because more (or less) hormone is produced than in a typical system or because the hormone is degraded faster (or slower) than is normal. Other EDCs influence transport of hormone, which can also change the amount of hormone that is available for receptor binding. And EDCs can also have effects that are independent from known endocrine actions. One example is the effect of endogenous hormones and EDCs on ion channel activity. BPA, dichlorodiphenyltrichloroethane (DDT), DES, nonylphenol, and octylphenol have all been shown to disrupt Ca²⁺ channel activity and/or Ca²⁺ signaling in some cell types (131–134). This example illustrates how both natural hormones and EDCs can have hormonal activity via binding to nuclear hormone receptors but may also have unexpected effects via receptor-mediated actions outside of the classical endocrine system.

2. Mechanisms of EDC-induced low-dose actions

The various mechanisms by which EDCs act *in vitro* and *in vivo* provide evidence to explain how these chemicals induce effects that range from altered cellular function, to abnormal organ development, to atypical behaviors. Just as natural hormones display nonlinear relationships between hormone concentration and the number of bound receptors, as well as between the number of bound receptors and the maximal observable biological effect, EDCs obey these rules of binding kinetics (38). Thus, in a way, EDCs exploit the highly sensitive endocrine system and produce significant effects at relatively low doses.

To gain insight into the effects of natural hormones and EDCs on gene expression profiles, it is possible to calculate doses that produce the same effect on proliferation of cultured cells, *i.e.* the quantitative cellular response doses, and determine the effect of those doses on transcriptomal signature profiles. When this is done for estradiol and EDCs with estrogenic properties, the affected estrogen-sensitive genes are clearly different (135). However, an interesting pattern emerges: comparing profiles among only the phytoestrogens shows striking similarities in the genes up- and down-regulated by these compounds; profile comparisons between only the plastic-based estrogens also show similarities within this group. Yet even more remarkable is what occurs when the doses are selected not based on cell proliferation assays but instead on the ability of estradiol and estrogen-mimics to induce a single estrogen-sensitive marker gene. When doses were standardized based on marker gene expression, the transcriptomal signature profiles were very similar between estradiol and estrogen mimics (135). Taken together, these results suggest that the outcomes of these experiments are contextual to the normalization parameter and that marker gene expression and cell proliferation are not superimposable. This indicates that the biological level at which the effects of chemicals are examined (*i.e.* gene expression, cellular, tissue, organ, or organismal) can greatly impact whether low-dose effects are observed and how these effects are interpreted.

There are several other mechanisms by which low-dose activities have been proposed. One such possibility is that low doses of EDCs can influence the response of individuals or organs/systems within the body to natural hormones; thus, the exposed individual has an increased sensitivity to small changes in endogenous steroids, similar to the effects of intrauterine position (see Ref. 136 and Section I.F). In fact, several studies have shown that exposure to EDCs such as BPA during perinatal development can influence the response of the mammary gland to estrogen (137, 138) and the prostate to an estrogen-testosterone

mixture similar to the concentrations produced in aging men (139–142). There is also evidence that EDCs work additively or even synergistically with other chemicals and natural hormones in the body (143–145). Thus, it is plausible that some of the low-dose effects of an EDC are actually effects of that exogenous chemical plus the effects of endogenous hormone.

Finally, it should be noted that during early development, the rodent fetus is largely, but not completely (146), protected from estrogen via the binding activity of α -fetoprotein, a plasma protein produced in high levels by the fetal liver (147). Some estrogen-like EDCs, however, bind very weakly to α -fetoprotein, and therefore, it is likely that this protein does not provide protection to the fetus during these sensitive developmental periods (36, 148). Furthermore, because EDCs may not bind to α -fetoprotein or other high-affinity proteins in the blood (148–150) and can have a higher binding affinity to proteins like albumin (compared with natural estrogens) (36, 149), the balanced buffer system in place for endogenous hormones may be disturbed (Fig. 1A). Thus, whereas only a portion of endogenous hormones are bioavailable, the entirety of a circulating EDC could be physiologically active.

The effects of hormones and EDCs are dependent on dose, and importantly, low (physiological) doses can be more effective at altering some endpoints compared with high (toxicological) doses. There are many well-characterized mechanisms for these dose-specific effects including signaling via single *vs.* multiple steroid receptors due to nonselectivity at higher doses (30), receptor down-regulation at high doses *vs.* up-regulation at low doses (151, 152), differences in the receptors present in various tissues (153, 154), cytotoxicity at high doses (155), and tissue-specific components of the endocrine-relevant transcriptional apparatus (104, 105). Some of these factors will be addressed in *Section III.B* in the section dedicated to NMDRCs.

F. Intrauterine position and human twins: examples of natural low-dose effects

Hormones have drastically different effects at different periods of development. In a now classical *Endocrinology* paper, Phoenix and colleagues (156) showed that hormone exposures during early development, and in particular fetal development, had organizational effects on the individual, whereby the developing organs were permanently reorganized by exposure to steroids. Permanent, nonreversible masculinization of the developing body plan by androgen exposure *in utero* is an example. These organizational effects are in contrast to the effects of the same hormones, at similar or even

higher doses, on adults. The effects of steroids on individuals after puberty have been termed *activational*, because the effects on target organs are typically transient; withdrawal of the hormone returns the phenotype of the individual to the preexposed state (157), although this is not always the case (158).

One of the most striking examples of the ability of low doses of hormones to influence a large repertoire of phenotypes is provided by the study of intrauterine positioning effects in rodents and other animals. The rodent uterus in particular, where each fetus is fixed in position along a bicornate uterus with respect to its neighbors, is an excellent model to study how hormones released from neighboring fetuses (159) can influence the development of endocrine-sensitive endpoints (31). Importantly, differences in hormonal exposures by intrauterine position are relatively small (see Fig. 2) (160). Thus, even a small magnitude in differences of hormonal exposures is sufficient to generate effects on behavior, physiology, and development.

The earliest studies of intrauterine position compared behavioral characteristics of females relative to their position in the uterus (161–164); male behavior was also affected by intrauterine position (161, 165–167). Subsequent studies of intrauterine position showed that position in the uterus influenced physiological endpoints (157, 160–162, 168–174) as well as morphological endpoints in female rodents (160, 161, 163, 164, 175–177). Male physiology and morphological endpoints were similarly affected by intrauterine position (165, 167, 177–179).

The endocrine milieu of the uterine environment has been implicated in these effects because differences in hormonal exposure have been observed based on intrauterine position (Fig. 2). The production of testosterone in male mice starting at approximately d 12 of gestation allows for passive transfer of this hormone to neighboring fetuses (159, 160, 180). Thus, fetuses positioned between two male neighbors have slightly higher testosterone exposures compared with fetuses positioned between one male and one female or two female neighbors (168, 181–183). These data indicate that very small differences in hormone exposures during fetal development are capable of influencing a variety of endpoints, many of which become apparent only during or after puberty. Furthermore, small differences in hormone exposures may be compounded by other genetic variations such as those normally seen in human populations.

Intrauterine effects have been observed in animals with both large litters and singleton or twin births including ferrets, pigs, hamsters, voles, sheep, cows, and goats (136, 184, 185). But perhaps the most compelling evidence for intrauterine effects comes from human twin studies. Many

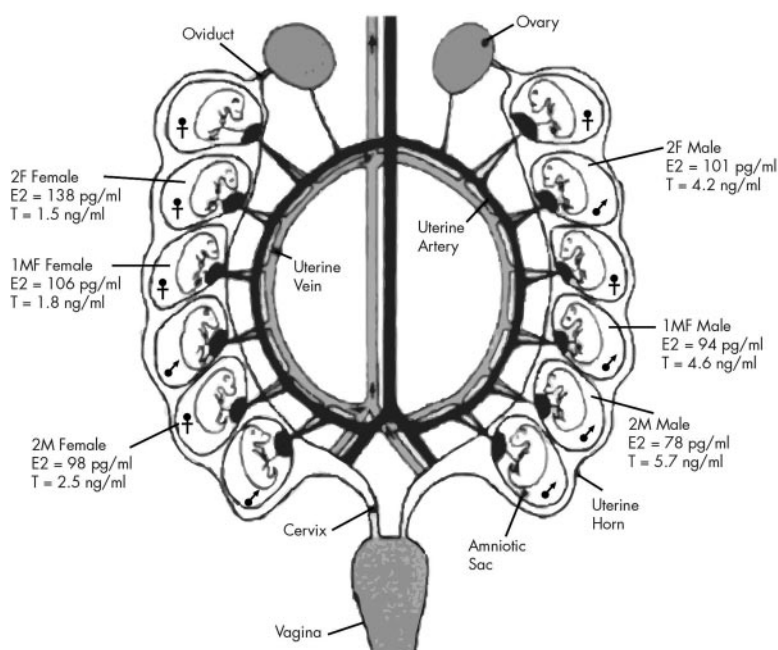
Figure 2.

Figure 2. Intrauterine position produces offspring with variable circulating hormone levels. Fetuses are fixed in position in the bicornate rodent uterus, thus delivery via cesarean section has allowed for study of the influence of intrauterine position on behaviors, physiology, and organ morphology. Illustrated here are the differences in estradiol (E2) and testosterone (T) concentrations measured in male and female fetuses positioned between two male neighbors (2M), two female neighbors (2F), or neighbors of each sex (1MF). Direction of blood flow in the uterine artery (dark vessel) and vein (light vessel) is indicated by an arrow (159).

studies have found that the sex of the fetuses impacts the phenotype of one or more of the twins, with significant evidence suggesting that male twins strongly influence a female co-twin; endpoints including sensation seeking (186), ear superiority (187, 188), brain and cerebellum volume (189), masculine/feminine behaviors and aggression levels (190–192), handedness (193, 194), reproductive fitness (192, 195), finger length ratios (196), risk for developing eating disorders (197), and birth weight (198) were all affected in females with a male twin. From these studies, many authors have concluded that testosterone from male fetuses influences developmental parameters in female twins; typically, male same-sex twins do not display altered phenotypes for these endpoints. Yet importantly, limited studies indicate that female twins can influence their uterine pairs, with some behaviors affected in male co-twins (191); breast cancer incidence in women and testicular cancer in men have also been shown to be influenced by having a female co-twin (83, 199, 200).

Although the mechanisms for these intrauterine effects are not completely understood, very small differences in hormone exposures have been implicated, making the effects of twin gestations a natural example of low-dose

phenomena. In the human fetus, the adrenals produce androgens that are converted to estrogen by the enzyme aromatase, specifically in the placenta. In a human study designed to compare hormone levels in the amniotic fluid, maternal serum, and umbilical cord blood of singleton male and female fetuses, significant differences were observed in the concentrations of testosterone, androstenedione (A4), and estradiol (201). Specifically, amniotic fluid concentrations of testosterone and A4 were approximately twice as high in male fetuses, whereas estradiol concentrations were slightly, but significantly, higher in female fetuses. Yet, interestingly, there were no differences for any of the hormones in maternal serum, similar to findings in mice that litters with a high proportion of males or females did not impact testosterone, estradiol, or progesterone serum levels in mothers (180). In umbilical cord serum, concentrations of A4 and estradiol were higher in males compared with females (201), although it must be noted that these samples were collected at parturition, long after the fetal period of sexual differentiation of the reproductive organs.

Several studies have specifically compared steroid hormone levels in maternal and umbilical cord blood samples collected from same-sex and opposite-sex twins. Male twins, whether their co-twin was a male or a female, had higher blood concentrations of progesterone and testosterone compared with female twins (202). Furthermore, for both sexes, dizygotic twins had higher levels of these hormones, as well as estradiol, compared with monozygotic twins. Fetal sex had no effect on maternal concentrations of testosterone, progesterone, or estrogen, suggesting that any differences observed in fetal samples are due to contributions from the fetuses' own endocrine systems and the placental tissue (203). Yet an additional study conducted in women carrying multiple fetuses (more than three) indicates that both estradiol and progesterone concentrations in maternal plasma increase with the number of fetuses, and when fetal reduction occurs, these hormone levels remain elevated (204).

It has been proposed that low-dose effects seen in different intrauterine positions in litter-bearing animals could be an evolutionary adaptation, whereby the genotypes of the fetuses are relatively similar but a range of phenotypes can be produced via differential hormone exposures (136, 168). For example, female mice positioned between two females are more docile and thus have better

reproductive success when resources are plentiful, but females positioned between two males are more aggressive and therefore are more successful breeders under stressful conditions (161, 171, 175). In this way, a mother produces offspring with variable responses to environmental conditions, increasing the chances that her own genetic material will continue to be passed on. Yet although there is evidence to suggest that a variable intrauterine environment is essential for normal development (171), intrauterine positional effects appear to have little effect on offspring phenotypes in inbred rodent strains (168, 205). This result may be related to the link between genetic diversity and hormone sensitivity (206, 207), suggesting that outbred strains are the most appropriate for studying endocrine endpoints and are also most similar to the effects of low doses of hormones on human fetuses.

Finally, it has been proposed that similar mechanisms are used by the developing fetus in response to natural hormones via intrauterine position and EDCs with hormonal activity (136). To this end, several studies have examined the effects of both exposure to an EDC and intrauterine position or have considered the effect of intrauterine position on the response of animals to these chemicals (174, 176, 181, 208, 209). For example, one study found that intrauterine position affected the morphology of the fetal mammary gland, yet position-specific differences were obliterated by BPA exposure (176). Additional studies suggest that prostate morphology is disrupted by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure in males positioned between two females, but this chemical does not affect prostate morphology in males positioned between two males (181). Finally, male rodents positioned between two males have higher glucose intolerance than males positioned between two females, yet when these males are given a diet high in phytoestrogens, glucose tolerance is dramatically improved in the males positioned between two males, whereas their siblings positioned between two females do not benefit (209). What is clear from these studies is that low doses of natural hormones are capable of altering organ morphology, physiology, and reproductive development, similar to the effects of EDCs.

It has been suggested that the endocrine system allows for homeostatic control and that the aim of the endocrine system is to “maintain normal functions and development in the face of a constantly changing environment” (210). Yet studies from intrauterine position, together with studies of EDCs (see *Sections II.C–F*), clearly indicate that the fetal endocrine system cannot maintain a so-called homeostasis and is instead permanently affected by exposures to low doses of hormones.

II. Demonstrating Low-Dose Effects Using a WoE Approach

A. Use of a WoE approach in low-dose EDC studies

In 2001, the NTP acknowledged that there was evidence to support low-dose effects of DES, genistein, methoxychlor, and nonylphenol (2). Specifically, the NTP expert panel found that there was sufficient evidence for low-dose effects of DES on prostate size; genistein on brain sexual dimorphisms, male mammary gland development, and immune responses; methoxychlor on the immune system; and nonylphenol on brain sexual dimorphisms, thymus weight, estrous cyclicity, and immune responses. Using the NTP’s definitions of low dose (*i.e.* effects occurring in the range of typical human exposures or occurring at doses lower than those typically used in standard testing protocols), we propose that most if not all EDCs are likely to have low-dose effects. Yet an important caveat of that statement is that low-dose effects are expected for particular endpoints depending on the endocrine activity of the EDC, and not for any/all endocrine-related endpoints. For example, if a chemical blocks the synthesis of a hormone, blood levels of the hormone are expected to decline, and the downstream effects should then be predicted from what is known about the health effects of low hormone levels. In contrast, if a chemical binds a hormone receptor, the effects are expected to be very complex and to be both tissue specific and dose specific. Finally, most EDCs interact with multiple hormone pathways, or even multiple hormone receptors, making the expected effects even more complex and context specific (211–213).

Table 3 summarizes a limited selection of chemicals that have evidence for low-dose effects, with a focus on *in vivo* animal studies. As seen by the results presented in this table, low-dose effects have been observed in chemicals from a number of classes with a wide range of uses including natural and synthetic hormones, insecticides, fungicides, herbicides, plastics, UV protection, and other industrial processes. Furthermore, low-dose effects have been observed in chemicals that target a number of endocrine endpoints including many that act as estrogens and antiandrogens as well as others that affect the metabolism, secretion, or synthesis of a number of hormones. It is also clear from this table that the cutoff for low-dose effects is not only chemical specific but also can be effect dependent. And finally, although this table is by no means comprehensive for all EDCs or even the low-dose effects of any particular chemical, the affected endpoints cover a large range of endocrine targets.

Several EDCs have been well studied, and the number of publications focusing on low-dose effects on a particular developmental endpoint is high; however, other

TABLE 3. EDCs with reported low-dose effects in animals (or humans, where stated)

Chemical	Use	EDC action	Low-dose cutoff	Affected endpoint	Refs.
Aroclor 1221 (PCB mixture)	Coolants, lubricants, paints, plastics	Mimics estrogens, antiestrogenic activity, etc.	0.1–1 mg/kg (produces human blood levels)	Brain sexual dimorphisms	683, 684
Atrazine	Herbicide	Increases aromatase expression	200 µg/liter (334, 335)	Male sexual differentiation/development	See this review
BPA	Plastics, thermal papers, epoxy resins	Binds ER, mER, ERRγ, PPARγ, may weakly bind TH receptor and AR	400 µg/kg · d (produces human blood concentrations)	Prostate, mammary gland, brain development and behavior, reproduction, immune system, metabolism	See this review
Chlordane	Insecticide	Binds ER	100 ng/g (produces human blood levels)	Sexually dimorphic behavior	685
Chlorothalonil	Fungicide, wood protectant	Aromatase inhibitor	164 µg/liter (environmental concentrations, EPA)	Corticosterone levels (amphibians)	686
Chlorpyrifos	Insecticide	Antiandrogenic	1 mg/kg · d (EPA)	Acetylcholine receptor binding (brain)	687
DDT	Insecticide	Binds ER	0.05 mg/kg (EPA)	Neurobehavior	688
DES	Synthetic hormone	Binds ER	0.3–1.3 mg/kg · d (dose typically administered to pregnant women)	Prostate weight	689
Dioxin (TCDD)	Industrial byproduct	Binds AhR	1 µg/kg · d (397)	Spermatogenesis, immune function and oxidative stress, tooth and bone development, female reproduction, mammary gland, behavior	See this review
Genistein	Phytoestrogen	Binds ER	50 mg/kg (EPA)	Brain sexual dimorphisms	690
Heptachlor	Insecticide	Induces testosterone hydroxylases	0.15 mg/kg · d (EPA)	Immune responses	691
Hexachlorobenzene	Fungicide	Modulates binding of ligand to TRE, weakly binds AhR	0.08 mg/kg · d (EPA)	Anxiety and aggressive behaviors	692
Maneb	Fungicide	Inhibits TSH release, may bind PPARγ	5 mg/kg · d (EU Commission)	Testosterone release	693
Methoxychlor	Insecticide	Binds ER	5 mg/kg · d (WHO)	Immune system	694, 695
4-Methylbenzylidene camphor	UV screen	Weakly estrogenic	10 mg/kg · d (Europa)	Sexual behavior	696
Methyl paraben	Preservative	Estrogenic	1000 mg/kg · d (EFSA)	Uterine tissue organization	697
Nicotine	Natural alkaloid in tobacco	Binds acetylcholine receptors, stimulates epinephrine	Human use of nicotine substitutes	Incidence of cryptorchidism (humans)	698
Nonylphenol	Detergents	Weakly estrogenic	15 mg/kg · d (EPA)	Testosterone metabolism	699
Octylphenol	Rubber bonding, surfactant	Weakly binds ER, RXR, PRGR	10 mg/kg · d (700)	Testes endpoints	701
Parathion	Insecticide		0.2 mg/kg · d (WHO)	Cognitive and emotional behaviors	702
PBDE-99	Flame retardant	Alters TH synthesis	0.3 mg/kg · d (EPA)	TH levels in blood	703
PCB180	Industrial lubricant, coolant	Impairs glutamate pathways, mimics estrogen	Examined normal human populations	Diabetes (humans)	704
PCB mixtures	Coolants, lubricants, paints, plastics	Binds AhR, mimic estrogens, antiestrogenic activity, etc.	Each at environmentally relevant levels	TH levels	705
Perchlorate	Fuel, fireworks	Blocks iodide uptake, alters TH	0.4 mg/kg · d (436)	TSH levels (humans)	See this review
Sodium fluoride	Water additive (to prevent dental caries), cleaning agent	Inhibits insulin secretion, PTH, TH	4 mg/liter water (EPA standard)	Bone mass and strength	706
Tributyltin oxide	Pesticide, wood preservation	Binds PPARγ	0.19 mg/kg · d (EPA)	Obesity	707
Triclosan	Antibacterial agent	Antithyroid effects, androgenic and estrogenic activity	12 mg/kg · d (Europe SCCP)	Altered uterine responses to ethinyl estradiol	708
Vinclozolin	Fungicide	Antiandrogenic	1.2 mg/kg · d (EPA)	Male fertility	709

EDC action indicates that for some chemicals, an effect is observed (*i.e.* estrogenic, androgenic), but for many EDCs, complete details of receptor binding are unavailable or incomplete. Low-dose cutoff means the lowest dose tested in traditional toxicology studies, or doses in the range of human exposure, depending on the data available. Affected endpoint means at least one example of an endpoint that shows significant effects below the low-dose cutoff dose. This list is not comprehensive, and the lack of an endpoint on this table does not suggest that low doses do or do not affect any other endpoints. AR, Androgen receptor; EFSA, European Food Safety Authority; ERR, estrogen related receptor; PCB, polychlorinated biphenyl; PPARγ, peroxisome proliferator-activated receptor-γ; PRGR, progesterone receptor; RXR, retinoid X receptor; SCCP, Scientific Committee on Consumer Products; TH, thyroid hormone; TRE, thyroid response element; WHO, World Health Organization.

chemicals are less well studied with fewer studies pointing to definitive low-dose effects on a given endpoint. In fact, there are a significant number of EDCs for which high-dose toxicology testing has been performed and the no observed adverse effect level (NOAEL) has been derived, but no animal studies in the low-dose range have been

conducted, and several hundred additional EDCs where no significant high- or low-dose testing has been performed (see Table 4 for examples). Balancing the large amount of data collected from some well-studied chemicals like BPA and atrazine with the relative paucity of data about other chemicals is a difficult task.

TABLE 4. Select examples of EDCs whose potential low-dose effects on animals remain to be studied

Chemical	Use	EDC action	Low-dose cutoff
Antiseptics and preservatives			
Butyl paraben	Preservative (cosmetics)	Estrogenic, antiandrogenic	2 mg/kg · d (EPA)
Propyl paraben	Antimicrobial preservative found in pharmaceuticals, foods, cosmetics, and shampoos	Estrogenic activity	LOAEL 10 mg/kg · d, NOEL 6.5 mg/kg · d (Europa)
Cosmetics and personal care products			
2,4-Dihydroxybenzophenone	UV absorber in polymers, sunscreen agent	Estrogenic activity	Not identified
3-Benzylidene camphor	UV blocker used in personal care products	Estrogenic activity	0.07 mg/kg · d (710)
4,4'-Dihydroxybenzophenone	UV light stabilizer used in plastics, cosmetics, adhesives, and optical fiber	Estrogenic activity	Not identified
Benzophenone-2	Used in personal care products such as aftershave and fragrances	Estrogenic activity, changes in T ₄ , T ₃ , and TSH levels, alterations in cholesterol profile	NOEL 10–333 mg/kg · d (711)
Benzophenone-3	UV filter	Estrogenic, PPAR γ activator	200 mg/kg · d (Europa)
Multiple use (other)			
Melamine	Flame-retardant additive and rust remover; used to make laminate, textile, and paper resins; metabolite of cyromazine	Affects voltage-gated K ⁺ and Na ⁺ channels and Ca ²⁺ concentrations in hippocampal neurons	63.0 mg/kg · d (FDA)
Resorcinol	Used in the manufacturing of cosmetics, dyes, flame retardants, hair dye formulations, pharmaceuticals, skin creams, and tires	Alters T ₄ and TSH levels	80.00 mg/kg · d (Europa)
Pesticides			
Aldrin ^a	Insecticide	Estrogenic activity	0.025 mg/kg · d (Health Canada)
Alachlor	Herbicide	Decreases serum T ₄ , binds PR, weakly binds ER	1 mg/kg · d (EPA)
Amitrole	Herbicide	Decreases thyroid hormone	0.12 mg/kg · d (FAO)
Bitertanol	Fungicide	Alters aromatase	30 mg/kg · d (EPA)
Carbendazim	Fungicide	Affects FSH, LH, and testosterone levels; alters spermatogenesis and Sertoli cell morphology	8 mg/kg · d (712)
Diazinon	Insecticide	Alters glucocorticoids	0.065 mg/kg · d (CDC)
Endrin ^a	Insecticide	Stimulates glucocorticoid receptor	0.025 mg/kg · d (CDC)
Fenoxycarb	Insecticide	Alters acetylcholinesterase	260 mg/kg · d (CDC)
Mirex ^a	Insecticide	Decreases testosterone levels	0.075 mg/kg · d (CDC)
Zineb	Fungicide	Alters T ₄ and dopamine levels	LOAEL 25 mg/kg · d (EPA)
Ziram	Fungicide	Alters norepinephrine levels	1.6 mg/kg · d (EPA)
Resins			
Bisphenol F	Used in polycarbonates	Alters T ₄ , T ₃ , and adiponectin levels, has estrogenic activity	LOAEL 20 mg/kg · d (713)
Styrene	Precursor to polystyrene	Alters dopamine	200 mg/kg · d (EPA)

PPAR γ , peroxisome proliferator-activated receptor- γ ; PR, progesterone receptor.

^a These chemicals were identified in the 1990s as part of the dirty dozen, 12 chemicals that were acknowledged to be the worst chemical offenders because of their persistence in the environment, their ability to accumulate through the food chain, and concerns about adverse effects of exposures to wildlife and humans. These chemicals were banned by the Stockholm convention and slated for virtual elimination. Yet there is still very little known about the low-dose effects of these chemicals, likely in the range of past and current human and/or wildlife exposures.

WoE approaches have been used in a large number of fields to determine whether the strength of many publications viewed as a whole can provide stronger conclusions than any single study examined alone. Although the term,

weight of evidence, is used in public policy and the scientific literature, there is surprisingly little consensus about what this term means or how to characterize the concept (214). Historically, risk assessors have used qualitative

approaches (*i.e.* professional judgment to rank the value of different cases) and quantitative approaches (*i.e.* scoring methods to produce statistical and mathematical determinations of chemical safety), but it has been argued that these methods lack transparency and may produce findings that are unrepeatable from one risk assessor to another (215, 216). Whatever the method used, when EDCs are being assessed, it is important to use the principles of endocrinology to establish the criteria for a WoE approach. We do this in *Section II.B*, identifying three key criteria for determining whether a study reporting no effect should be incorporated into a WoE approach. It also should be noted that in epidemiology, the term, weight of evidence, is typically not used, but the concept is actuated by meta-analysis, formally and quantitatively combining data across studies, including a plot of individual and pooled study findings and also a measure of heterogeneity of findings between studies.

For some well-studied chemicals, there are large numbers of studies showing both significant effects, and additional studies showing no effects, from low-dose exposures. In these cases, extensive work is needed to deal with discordant data collected from various sources; studies showing no effect of low-dose exposures must be balanced in some way with those studies that do show effects. As stated by Basketter and colleagues (217), “it is unwise to make a definitive assessment from any single piece of information as no individual assay or other assessment . . . is 100% accurate on every occasion . . . This means that from time to time, one piece of conflicting data has to be set aside.” WoE approaches in EDC research have typically dealt with datasets that have some conflicting studies, and these conflicts are even more difficult to sort out when studies have attempted to directly replicate published findings of adverse effects (see for example Refs. 218–221).

Most previously published WoE analyses have examined chemicals broadly (asking questions such as, “Does BPA produce consistent adverse effects on any endpoint?”) (see Ref. 222). This can lead to problems including those encountered by the NTP expert panel, which found that there was some evidence for low-dose effects of BPA on certain endpoints but mixed findings for other endpoints. For example, the panel noted that some studies found low-dose effects of BPA on the prostate, but other studies could not replicate these findings. In *Section II.B*, we address criteria that are needed to accept those studies that are unable to detect low-dose effects of chemicals; these criteria were not used by the NTP in 2001, but they are essential to address controversies of this sort and perform WoE analyses using the best available data. In the sections that follow, we employed a WoE approach to

examine the evidence for low-dose effects of single chemicals on selected endpoints or tissues, also paying attention to when in development the EDC in question were administered.

B. Refuting low-dose studies: criteria required for acceptance of studies that find no effect

Over the past decade, a variety of factors have been identified as features that influence the acceptance of low-dose studies (69, 71, 76, 77, 90, 205, 223, 224). In fact, the NTP low-dose panel itself suggested that factors such as strain differences, diet, caging and housing conditions, and seasonal variation can affect the ability to detect low-dose effects in controlled studies (2). In particular, three factors have been identified; when studies are unable to detect low-dose effects, these factors must be considered before coming to the conclusion that no such effects exist.

1. Negative controls confirm that the experimental system is free from contamination

Although all scientific experiments should include negative (untreated) controls, this treatment category is particularly important for EDC research. When a study fails to detect low-dose effects, the observed response in control animals should be compared with historical untreated controls; if the controls deviate significantly from typical controls in other studies, it may indicate that these animals were, in fact, treated or contaminated in some way or that the endpoint was not appropriately assessed (77, 205, 225). For example, if an experiment was designed to measure the effect of a chemical on uterine weight, and the control uteri have weights that are significantly higher than is normally observed in the same species and strain, these animals may have been inadvertently exposed to an estrogen source, or the uteri may not have been dissected properly by the experimenters. In either case, the study should be examined carefully and likely cannot be used to assess low-dose effects; of course, untreated controls should be monitored constantly because genetic drift and changes in diet and housing conditions can also influence these data, thus explaining changes from historical controls. Importantly, several types of contamination have been identified in studies of EDCs including the leaching of chemicals from caging or other environmental sources (226, 227), the use of pesticide-contaminated control sites for wildlife studies and contaminated controls in laboratory studies (76), and even the use of food that interferes with the effects of EDCs (224, 228). It is also important to note that experiments must consider the solvent used in the administration of their test chemical, and thus good negative controls should test for effects of the solvent itself. Using solvent negative controls helps prevent false posi-

tives as well as the possibility that the vehicle could mask the effects of the chemical being studied.

2. Positive controls indicate that the experimental system is capable of responding to low doses of a chemical acting on the same pathway

Many studies do not include a positive control, either because of the size and cost of the experiment when including an additional treatment or because an appropriate positive control has not been identified for the endpoint being examined. If the experiment detects an effect of the chemical in question, the exclusion of a positive control does not necessarily affect the interpretation of the results; instead, it can be appropriately concluded that the test chemical is significantly different from unexposed (but similarly handled/treated) negative controls. However, if the study fails to detect low-dose effects of a test chemical, no convincing conclusion can be made; in this case, a positive control is required to demonstrate that the experimental system was capable of detecting such effects (71, 75, 77, 205).

Several issues must be considered when addressing whether the positive control confirms the sensitivity of the assay. First, an appropriate chemical must be selected, and it must be administered via the appropriate route, *i.e.* if the test chemical is administered orally, a positive control that is orally active, such as ethinyl estradiol, should be used; if the test chemical is administered *sc*, a positive control that is active via this route, such as 17β -estradiol, is most appropriate. The use of 17β -estradiol in studies that use oral exposures is particularly inappropriate (see Ref. 229) for example) because this hormone, like most natural steroids, has very low oral activity (77). Second, the positive control chemical must be examined, and effective, at appropriately low doses. Thus, if the test chemical is 100 times less potent than the positive control, a dose of the positive control 100 times lower than the test compound must produce effects (69, 71, 205). For example, studies that report effects of ethinyl estradiol only at doses that are hundreds of times higher than the dose that is effective in contraceptives (230) are not capable of detecting low-dose effects of test chemicals. Without appropriate and concurrent positive and negative controls, studies that fail to detect low-dose effects of test chemicals should be rejected.

3. Species and animal strains that are responsive to EDCs must be used

The NTP expert panel specifically noted that “because of clear species and strain differences in sensitivity, animal-model selection should be based on responsiveness to endocrine-active agents of concern (*i.e.* responsive to pos-

itive controls), not on convenience and familiarity” (2). An analysis of the BPA literature clearly showed that many of the studies that failed to detect effects of low doses used the Charles River Sprague-Dawley rat (75); this strain was specifically bred to have large litters (231), and many generations of inbreeding have rendered the animal relatively insensitive to estrogens (205). The NTP expert panel noted the lack of effects of BPA on Sprague-Dawley rats and concluded that there were clear differences in strain sensitivity to this chemical (2). Importantly, this may not be true for Sprague-Dawley rats that originate from other vendors, indicating that animal origin can also influence EDC testing.

Many studies in mice (138, 206, 207, 232–234) and rats (232, 235–239) have described differences displayed between two (or more) animal strains to a natural hormone or EDC. Often these differences can be traced to whether a strain is inbred or outbred. Genetically diverse strains are generally found to be more sensitive to estrogens (206). Importantly, well-controlled studies demonstrate that strain differences in response to estrogen treatment may be organ dependent or may even differ between levels of tissue organization within the same organ. For example, the Sprague-Dawley rat is more sensitive to ethinyl estradiol than other strains when measured by uterine wet weight. However, when other endpoints were measured, *i.e.* height of cells in the uterine epithelium, the Sprague-Dawley rat was indistinguishable from the DA/Han rat; instead, the Wistar rat had the most heightened response (237). Additionally, there are data to indicate that strain differences for one estrogen may not be applicable for all estrogenic chemicals. In comparing the responses of DA/Han, Sprague-Dawley, and Wistar rats to other xenoestrogens, additional differences were observed including a greater increase in uterine wet weight of DA/Han and Sprague-Dawley rats but not Wistar rats after exposure to 200 mg/kg BPA; increased uterine epithelium thickness was observed in Wistar and Sprague-Dawley rats but not DA/Han rats after exposure to 200 mg/kg octylphenol (237). Attempts have been made, at times successfully, to map the differences in strain response to genetic loci (240). However, it appears that strains with differences in response that manifest in some organs do not have divergent responses in other organs, a phenomenon that is not explained by genetic differences alone. For these reasons, the NTP’s recommendation that scientists use animals that are proven responsive to EDCs (2) must be observed.

4. Additional factors?

Additional factors have also been identified as influential in the ability (or inability) to detect low-dose effects in

EDC studies. Although these factors must be considered when interpreting studies and using a WoE approach, some issues that were previously identified as essential factors in the design of studies (*i.e.* route of administration) have more recently been disputed (241).

The first factor is the use of good laboratory practices (GLP) in the collection of data. When assessing the EDC literature for risk assessment purposes, the FDA and European Food Safety Authority (EFSA) have given special prominence to studies that complied with GLP guidelines, essentially giving scientific priority to industry-funded studies because that group typically conducts GLP guideline studies (33, 242). Because GLP guidelines are designed only to control data collection, standards for animal care, equipment, and facility maintenance, and they do not ensure that studies were designed properly with the appropriate controls, it has been argued that the use of GLP methods is not appropriate or required for EDC studies (69).

GLP studies are typically large, with dozens of animals studied for each endpoint and at each time point. Thus, it has been concluded that these studies are better simply because they are larger. Yet small studies designed with the use of power analysis, statistical tools that allow researchers to determine *a priori* the number of animals needed to determine significant differences based on effect size, are equally capable of detecting effects while reducing the number of animals used (69). GLP studies also typically (but not necessarily) rely upon standardized assays, which are not generally considered contemporary tools and are often shown to be incapable of detecting adverse effects on endpoints that employ modern tools from molecular genetics and related disciplines. Furthermore, some fields of EDC research have no GLP studies (243). Finally, there is no published evaluation of whether studies performed under GLP are more capable of providing accurate results. The priority given to GLP studies therefore does not appear to have been justified based on any comparative analysis. Thus, as long as studies include appropriate measures of quality assurance, they need not be performed under GLP standards to provide reliable and valuable information, and many GLP studies are inadequate to assess important and relevant endpoints. Instead, the most valuable studies consider the factors presented above, along with appropriate dose selections and choice of endpoint.

The second factor worth considering is the source of funding for studies. In several fields, significant controversy has been produced based on the results obtained from independent scientists compared with results obtained from scientists affiliated with the chemical industry (75, 76). Funding source *per se* should not dictate the outcome of a research study, but that does not mean that

researchers are not subject to underlying biases. In our own WoE analyses, presented in *Sections II.C–G*, we do not discount studies merely because they were conducted with industry funds, nor do we lend higher weight to studies conducted in independent or government laboratories; if a study, regardless of funding, finds no effect of a chemical, it is given weight only if the three criteria described in *Sections II.B.1–3* (successful and appropriate negative and positive controls and appropriate choice of animal model) were met.

To perform a WoE evaluation, we identified some basic information about the chemical in question, the dose that would be considered a low-dose cutoff, and the studies in support of and against low-dose effects. We then considered whether the majority of studies found effects of low doses of a chemical on a single endpoint in question. If studies did not find low-dose effects, we considered whether they adhered to the criteria discussed above for proper design of an EDC low-dose study. In particular, we considered whether appropriate animal strains as well as positive and negative controls were used. With regard to animal strain, as discussed briefly in *Section II.B.3*, there is variability between animal strains that can significantly influence the ability to detect effects of EDCs; using insensitive strains to produce negative data cannot refute positive data in a sensitive strain. In several cases, it was easy to conclude that there was a strong case for low-dose effects because there were no studies finding no effects at low doses or because all of the negative studies were inappropriately designed. For other chemicals, a significant number of studies found effects on the endpoint being considered, but other (adequately designed) studies refuted those findings. Under those circumstances, we determined whether the findings of harmful effects came from multiple laboratories; when they did, we cautiously concluded that there was evidence for low-dose effects. Below (*Sections II.C–G*), we present five examples where a significant number of studies were available examining low-dose effects of an EDC on a single particular endpoint.

C. BPA and the prostate: contested effects at low doses?

As discussed briefly above, BPA is one of the best-studied EDCs, with more than 200 published animal studies, many of which focused on low doses (29, 31). The effects of this chemical on wildlife species have also been described in detail (28). BPA is found in a myriad of consumer products, and it leaches from these items under normal conditions of use (4). It has also been regularly detected in air, water, and dust samples. The majority of individuals in industrialized countries have BPA metabolites in their urine, and trends indicate increasing expo-

tures in developing nations like China (87, 244). Although it was long suspected that most human exposures originate from BPA contamination of food and beverages, a study comparing the excretion of BPA metabolites with the length of time spent fasting suggests that there are also likely to be significant exposures from sources other than food and beverages (245). BPA has recently been shown to be used in large quantities in thermal and recycled papers and can enter the skin easily via dermal absorption (246–248). Thus, despite the large amount of information available on BPA sources, our understanding of how these sources contribute to total human exposures remains poor; these studies also point to significant gaps in current knowledge about BPA metabolism in humans (243).

BPA binds to the nuclear and membrane ER, and thus most of the effects of this chemical have been attributed to its estrogenic activity (27). However, there is evidence that it can activate a number of additional pathways, including thyroid hormone receptor, androgen receptor, as well as peroxisome proliferator-activated receptor- γ signaling pathways (249–252). The cutoff for a low dose has been set at several different concentrations depending on which studies and definitions are used (see Table 1). The EPA calculated a reference dose for BPA of 50 $\mu\text{g}/\text{kg} \cdot \text{d}$ based on a LOAEL of 50 $\text{mg}/\text{kg} \cdot \text{d}$ (38). More recent pharmacokinetic scaling experiments have estimated that exposures to approximately 400 $\mu\text{g}/\text{kg} \cdot \text{d}$ produce blood concentrations of unconjugated BPA in the range of human blood concentrations (4). Thus, for the two WoE analyses of the BPA literature we conducted, doses of 400 $\mu\text{g}/\text{kg} \cdot \text{d}$ or lower were considered low dose; pharmacokinetic studies from nonhuman primates support the appropriateness of this dose for approximating human exposure levels (253). Furthermore, because this dose is below the toxicological LOAEL, it is a conservative cutoff for low-dose studies (see Refs. 3 and 38 and Table 1).

One of the most well studied and hotly debated examples of a low-dose effect comes from the BPA literature; regulatory agencies and scientists have addressed several times whether low doses of BPA during fetal and perinatal development affect the rodent prostate (118, 205, 254, 255). In 1997, the first study on BPA and the prostate determined that fetal exposure to low doses (2 and 20 $\mu\text{g}/\text{kg} \cdot \text{d}$ administered orally to pregnant mice) increased the weight of the adult prostate compared with unexposed male offspring (256). Since that time, several additional studies have verified that prostate weight is affected by fetal exposure to similar low doses (257–259). Studies have also shown that low doses of BPA affect androgen receptor binding activity in the prostate (257), tissue organization, and cytokeratin expression in the gland (260–262) as well as the volume of the prostate and the number

and size of dorsolateral prostate ducts (208). Several recent studies have also examined whether low doses of BPA (10 $\mu\text{g}/\text{kg} \cdot \text{d}$) influence the incidence of adult-onset prostatic intraepithelial neoplasia (PIN) lesions. Perinatal BPA exposure, whether administered orally or sc to pups, increases the incidence of PIN lesions in response to a mixture of testosterone and estradiol in adulthood (139, 141, 263); this hormonal cocktail was designed to mimic the endocrine changes associated with aging in men that also typically accompany the onset of prostate cancer. In addition to the effects of BPA on PIN lesions, these low doses also produced permanent alterations in the epigenome of exposed males, with prostates displaying completely unmethylated sequences in genes that are hypermethylated in unexposed controls (140, 263). In examining these studies, although the same effects of BPA on the prostate were not observed in all studies, there is an obvious trend demonstrating that low doses of BPA during early development significantly affect several aspects of prostate development.

Since the initial report showing effects of low doses on the prostate, approximately nine studies, including several designed specifically to replicate the original positive study, have shown no effects of low doses on the prostate (264–272); every one of these studies examined the prostate weight, and Ichihara *et al.* (264) also examined the effects of BPA on PIN lesions (without hormonal treatment) and the response of the prostate to a chemical carcinogen. Three of these studies failed to include a positive control of any kind (264, 268, 270); three studies used DES as a positive control but found no effect from exposure to this potent xenoestrogen (265–267) (*i.e.* the positive control failed); another study used 17 β -estradiol as a positive control, inappropriately administered orally, and found no effects of this hormone on the prostate (271); and two studies used an estrogenic positive control (ethinyl estradiol) and found effects from its exposure, but only at inappropriately high doses (269, 272). These two studies clearly showed that the positive control dose was too high, because rather than increase the weight of the prostate (as seen after low doses of estrogens in other studies), the positive control decreased the weight of the adult prostate (269, 272).

Although this topic was once considered controversial, using a WoE approach, it is clear that there is strong evidence in support of low-dose effects of BPA on the development of the prostate. The evidence clearly shows that several endpoints, including prostate weight, were affected in similar ways in multiple studies from several different labs at doses below 400 $\mu\text{g}/\text{kg} \cdot \text{d}$; most effects were seen at doses below 50 $\mu\text{g}/\text{kg} \cdot \text{d}$. Furthermore, PIN lesions were reported after neonatal exposure to 10 $\mu\text{g}/\text{kg} \cdot \text{d}$ with

hormonal treatment in adulthood. No appropriately conducted studies contest this evidence. Therefore, the WoE analysis demonstrates that low doses of BPA significantly alter development of the rodent prostate. The NTP's review of the BPA literature in 2008 indicated that this agency agrees that there is now significant evidence that low-dose BPA adversely affects development of the prostate (273).

D. BPA and the mammary gland: undisputed evidence for low-dose effects

The mammary gland is a conspicuous choice to examine the effects of estrogenic compounds because this organ depends on estrogen for proper development at several critical periods in life (274). The fetal gland expresses ER in the mesenchymal compartment, and just before birth, the epithelium becomes ER positive as well (275). At puberty, estrogen is responsible for ductal elongation and overall development of the gland, allowing the epithelium to fill the stromal compartment in preparation for pregnancy and lactation. Although BPA is an example of a chemical that has been classified as a weak estrogen because it binds with a much lower affinity to ER α compared with 17 β -estradiol, even weak estrogens are known to affect the development of the mammary gland during early development (276).

In the first study to examine the effects of BPA on the mammary gland, prepubertal rats were exposed to relatively high doses (100 $\mu\text{g}/\text{kg} \cdot \text{d}$ or 54 $\text{mg}/\text{kg} \cdot \text{d}$) for 11 d. After even this short exposure, mammary gland architecture was affected in both dose groups, with increased numbers of epithelial structures and, in particular, structures that suggest advanced development (277). BPA exposure also altered proliferation rates of mammary epithelium and cell cycle kinetics, with an increased number of cells in S-phase and a decreased number of cells in G1. Although relatively high doses of BPA were examined, this initial study indicated that the prepubertal and pubertal gland could be sensitive to BPA.

Many additional studies have examined another critical period, the fetal and neonatal periods, which are sensitive to environmental estrogens (78, 276, 278). Mice exposed prenatally to low doses of BPA via maternal treatment (0.25 $\mu\text{g}/\text{kg} \cdot \text{d}$) displayed altered development of both the stromal and epithelial compartments at embryonic d 18, suggesting that exposures affect tissue organization during the period of exposure (176). In addition, similar low doses produced alterations in tissue organization observed in puberty and throughout adulthood, long after exposures ended, and even induced pregnancy-like phenotypes in virgin females (137, 279–282). Female mice exposed to BPA *in utero* displayed heightened re-

sponses to estradiol at puberty, with altered morphology of their glands compared with animals exposed to vehicle *in utero* (138). Another study demonstrated that perinatal BPA exposure altered the mammary gland's response to progesterone (283). Remarkably, all of these effects were observed after maternal exposures to low doses (0.025–250 $\mu\text{g}/\text{kg}$), suggesting that the gland is extremely sensitive to xenoestrogen exposures. These studies are in contrast to one that examined the effects of higher doses (0.5 and 10 $\text{mg}/\text{kg} \cdot \text{d}$) when BPA was administered for 4 d to the dam, which reported advanced development of BPA-exposed glands before puberty but no effects in adulthood (284).

Adult exposure to BPA is only now being examined in the mouse mammary gland model. A recent study examined the effects of BPA on mice with mutations in the *BRCA1* gene. This study reported that 4 wks of exposure to a low dose of BPA altered the tissue organization of the mammary gland in ways that are similar to the effects observed after perinatal exposure (285). This study focused on altered development of the gland during exposure; additional studies are needed to determine whether these effects are permanent or whether normal mammary morphology could be achieved by cessation of BPA exposure.

Another obvious endpoint is the effect of BPA exposure on mammary cancer incidence. Several studies indicate that exposure to BPA *in utero* produces preneoplastic (281, 286, 287) and neoplastic lesions (286) in the gland in the absence of any other treatment. Additionally, other studies show that females exposed to BPA during the perinatal period are more sensitive to mammary carcinogens, decreasing tumor latency and increasing tumor incidence (287–290). These studies are also supported by subsequent studies examining gene and protein expression, which show that low-dose BPA specifically up-regulates expression of genes related to immune function, cell proliferation, cytoskeletal function, and estrogen signaling and down-regulates apoptotic genes (282, 288, 289, 291).

Postnatal BPA exposures also influence mammary cancer incidence; animals exposed lactationally to BPA from postnatal d 2 until weaning displayed decreased tumor latency and increased tumor multiplicity after treatment with DMBA [7,12-dimethylbenz(a)anthracene], a carcinogen (292). This study suggested that BPA exposure led to increased cell proliferation and decreased apoptosis in the gland and shifted the period where the gland is most susceptible to mammary carcinogens, a result that has important implications for human breast cancer. Finally, an additional study examined the effects of adult BPA exposure on mammary cancer; this study demonstrated that low doses of BPA accelerate the appearance of mammary tumors in a tumor-prone mouse strain (293). Interestingly,

high doses did not have this effect; thus, this study is also an excellent example of a NMDRC.

Two studies of BPA and the mammary gland seem to contradict this body of literature, but both examined extremely high doses. In the first study, Nikaido *et al.* (294) exposed female mice to 10 mg/kg BPA from postnatal d 15–18. Mammary glands from these animals were examined at 4, 8, and 24 wk of age, and no differences were observed in the exposed animals relative to controls. Although the lack of effects reported in this study could be due to the high dose employed, they could also be related to the relatively short exposure period during the preweaning phase. In the second study, Yin and colleagues (295) examined the effects of BPA during the first few days after birth (0.1 or 10 mg BPA, equivalent to approximately 10 and 1000 mg/kg) on the incidence of mammary tumors after exposure to a mammary carcinogen at puberty. Similar to the study described above, this one also examined the effects of BPA after a relatively short period of exposure (only three injections administered between postnatal d 2 and 6). Although the study showed that BPA affected tissue organization, there was no change in the incidence of tumors in BPA-exposed females. Because both of these studies examined both high doses and relatively short periods of exposure, it is difficult to compare them directly to the studies finding effects of BPA on the mammary gland after longer exposures to lower doses; at the very least, they cannot refute studies suggesting that BPA alters development of this gland.

In summary, the WoE clearly shows that low-dose BPA exposure affects development of the mammary gland, mammary histogenesis, gene and protein expression in the gland, and the development of mammary cancers. In fact, this example of low-dose effects produced remarkably similar effects across more than a dozen studies conducted in several different labs. These results are also consistent with the effects of low-dose BPA exposure on mammary epithelial cells in culture (reviewed in Ref. 30). Although epidemiology studies examining the influence of BPA on breast cancer rates have proven to be inconclusive at best (296), to replicate the animal studies discussed above, epidemiologists must collect information about prenatal and neonatal exposures and relate them to adult breast cancer incidence. These types of studies would take decades to conduct (67) and should take into consideration the effects of other estrogens, because their effects can be additive or even synergistic (143, 144, 297).

Although our analyses of BPA have focused on its effects on the mammary gland and prostate (see *Sections II.C–D*), it is worth noting that several other endpoints have strong data to support the hypothesis that BPA has low-dose effects. In a recent review using similar WoE

approaches, Hunt and colleagues (298) focused on those studies that examined the effects of BPA on the oocyte, specifically scrutinizing studies that reported effects, or no effects, on meiotic aneuploidy and other alterations in the intracellular organization and chromosome abnormalities. Similar to what has been observed with the prostate and mammary gland, the effects observed in the oocyte are variable from study to study, but overall consistent, and suggest that BPA exposure produces defects in these cells.

A large number of studies have also focused on the effects of BPA on the brain and behavior, with the most significant effects on sexually dimorphic regions of the brain and behaviors (299–307). Other affected behaviors include social behaviors, learning and anxiety, and maternal-neonate interactions (reviewed in Refs. 29 and 308). The NTP expert panel statement concluded that there were significant trends in these behavioral data and wrote that there was some concern that BPA could have similar effects in humans (273). Low-dose effects have also been reported for BPA in the female reproductive tract (309, 310), immune system (311, 312), maintenance of body weight and metabolism (313, 314), fertility (315–317), and the male reproductive tract (259, 318) (see Refs. 29 and 319 for comprehensive reviews).

E. Another controversial low-dose example: atrazine and amphibian sexual development

Atrazine is an herbicide that is applied in large volumes to crops, and there is concern that agricultural runoff of this chemical can affect nontarget animal species, especially amphibians that live and reproduce in small ponds and streams where significant amounts of atrazine have been regularly measured (320–322). It is the most commonly detected pesticide in ground and drinking water. Atrazine induces aromatase expression in cells and animals after exposure (323); this ultimately causes an increase in the conversion of testosterone to estrogen (324, 325). This effect has been reported in all vertebrate classes examined: fish, amphibians, reptiles, birds, and mammals, including human cell lines (see Ref. 326 for review). Another well-documented effect of atrazine is that it decreases androgen synthesis and activity, again, in every vertebrate class examined (326). In addition, endocrine-disrupting effects of atrazine occur through a number of other mechanisms, including antiestrogenic activity (327), altered prolactin release (328), and increased glucocorticoid release from the adrenal glands (329, 330), among others (327).

Because of atrazine's indirect effect on estrogen levels, one relevant endpoint that has been given attention is the effect of this chemical on gonad differentiation in various amphibian species. The early gonad is bipotential, and in

mammals, the expression of genes on the Y-chromosome is needed to masculinize the undifferentiated gonad; when this does not occur, the gonad develops into ovarian tissue. In *Xenopus laevis* frogs (and some other animals like birds), the opposite is true: females are heterogametic (*i.e.* ZW-chromosomes) and males have two of the same chromosomes (*i.e.* ZZ). In *X. laevis*, the W-chromosome is the dominant one, containing a gene, DM-W, which induces aromatase expression (331). Thus, having a W-chromosome is needed to produce estrogen; without the conversion of testosterone to estrogen, the frog develops as a male (332). Changes in sex ratio and gonadal morphology are therefore good indicators that an estrogen, or a chemical that up-regulates aromatase and indirectly increases estrogen levels, is present (76).

Determining a low-dose cutoff for atrazine is not a simple task. Although the safe limit of 3 $\mu\text{g}/\text{liter}$ in drinking water was set by the EPA, actual levels in the environment often exceed this concentration (333), and levels in ponds and streams can reach 100 $\mu\text{g}/\text{liter}$ (322) or more. In traditional toxicology studies examining several amphibian species, the LOAEL was set at 1.1 mg/liter, and the no observed effect level (NOEL) was 200 $\mu\text{g}/\text{liter}$ (334, 335). Thus, using the definitions of low dose established by the NTP (2), we consider any treatment at or below 200 $\mu\text{g}/\text{liter}$ to be a low dose.

In 2002, one of the first published studies to connect atrazine exposures to altered gonadal morphology examined *X. laevis* frogs exposed to 0.01–200 $\mu\text{g}/\text{liter}$ throughout larval development (336). All doses from 0.1–200 $\mu\text{g}/\text{liter}$ produced gonadal malformations including the presence of multiple gonads and hermaphroditism. Several other reports showed similar effects of low doses on gonadal phenotypes including studies that report the production of hermaphrodites and intersex frogs, males with ovotestes, and males with testicular oocytes (337–343). Additional studies showed that low-dose atrazine exposure (0.1–200 $\mu\text{g}/\text{liter}$ in the water) during sexual differentiation caused testicular dysgenesis, testicular resorption, and testicular aplasia in male frogs (343, 344), and others indicated effects on sex ratios (339, 342, 345, 346). Importantly, these effects were not all observed at the same atrazine concentration, and the studies were conducted in several different species, with some reporting effects at low doses but no effects at higher doses (341) and others reporting effects in some but not all species (339). Examining these studies as a whole, there is clearly a pattern of effects that are reproducible from study to study, and they collectively support the hypothesis that atrazine disrupts sex hormone concentrations.

To date, five peer-reviewed studies have reported no effects of atrazine on sex ratios, gonadal morphology, the

incidence of testicular abnormalities or testicular oocytes, gonad size, or the incidence of intersex phenotypes (347–351). Little can be ascertained from these negative studies, however, because four did not include any positive control, suggesting that the frogs used in those studies may have been incapable of responding to atrazine or any other hormonal treatment (347–350). Additionally, one of those studies reported testicular oocytes in the control frogs, suggesting either that the negative control population was contaminated with atrazine (or another EDC or hormone), or that an inappropriate strain of *X. laevis* was selected for the experiments (347). Only one study remains that did not find any effects of atrazine; this study used an appropriate positive control (17 β -estradiol) and found effects of that hormone on sex ratios and the incidence of intersex gonads (351). An EPA expert panel noted, however, that this study used a strain of *X. laevis* that was obtained from a new, unexamined population of frogs from Chile and suggested that this strain may be insensitive to environmental chemicals. Furthermore, the panel called for additional analysis of the data in this study, including the statistical approaches; they suggested that an independent laboratory should evaluate the histopathological results; and they requested that atrazine metabolites be measured (352). The panel also proposed that these experiments should be repeated with an established *X. laevis* strain. Taking together the results of those studies that found effects of atrazine on sexual differentiation, and this one negative study, the WoE for the case of low-dose atrazine on sexual differentiation is clearly in support of adverse effects of this chemical.

Just as epidemiological studies have found links between EDCs and human diseases, ecological field studies have examined whether exposure to atrazine in natural environments affects the development of wild amphibians (343, 353–358). These studies have many of the same constraints as those observed in epidemiology: a paucity of data on early life exposures (including exposure levels of controls), limitations on the total number of EDCs that can be measured in environmental and biological samples, and a lack of causative relationships that can be established between exposures and effects. For these reasons, studies that found relationships between atrazine exposure (or concentrations in environmental samples) and effects on one or more aspect of sexual differentiation (343, 353–355) are considered weak, but significant, evidence for low-dose effects. The presence of several studies suggesting a relationship between low-dose exposure to atrazine in the wild and altered sexual differentiation indicates a plausible causal relationship. Because the ecological and laboratory data show similar effects of atrazine on go-

nadal development, this strengthens the conclusions of our WoE that low doses of atrazine cause harm to amphibians.

Feminization of males after atrazine exposure is not restricted to amphibians; exposure of zebrafish to low doses increased the ratio of female to male fish and increased expression of aromatase (359). Close to a dozen additional studies also report that environmentally relevant doses of atrazine can up-regulate aromatase, decrease testosterone, and/or increase estrogen levels in a large number of species (reviewed in Ref. 119), suggesting that low-dose effects of atrazine may be more widespread than their effects on the gonads of amphibians. Other studies indicate that low-dose atrazine affects the immune system and stress responses of salamanders (360–362), survivorship patterns of several frog species (363), and thyroid hormone and plasma ion concentrations in salmon (364).

An important factor to consider when examining the effects of atrazine on different animal models is the difficulty in identifying an appropriate low, environmentally relevant dose for all species. Aquatic animals can be housed in water containing levels of atrazine found in wild habitats, yet no toxicokinetic studies are available to determine what administered dose produces the levels of atrazine metabolites, typically in the parts-per-million or ppb range (365, 366), measured in human samples. There are also no blood or urine measurements in exposed rodents to compare with human levels; thus, extrapolations across species are estimates at best.

Keeping this qualification in mind, exposures in the range of 25–100 mg/kg · d during development have been shown to alter mammary gland development (367, 368), estrous cyclicity (369), serum and intratesticular testosterone concentrations (370), timing of puberty in males and prostate weight (371), and immune function (372) in rodents. Lower doses of atrazine metabolites (0.09–8.73 mg/kg · d) altered development of the mammary gland (373), male pubertal timing and prostate development (374). Identifying the range of doses administered to animals that produce the levels of atrazine and its metabolites measured in human blood and urine is an essential research need to pursue low-dose studies in rodents and other mammals.

F. Dioxin and spermatogenesis: low-dose effects from the most potent endocrine disruptor?

Dioxin, or TCDD, is formed as a byproduct of industrial processes as well as during waste incineration. Because TCDD is extremely toxic to some animals, with 1 $\mu\text{g}/\text{kg}$ capable of killing 50% of guinea pigs, it has been labeled the most toxic chemical on earth (375). But interestingly, other animals are less sensitive to lethal effects of TCDD, with an LD₅₀ of approximately 1000 $\mu\text{g}/\text{kg}$ in

hamsters, and studies also suggest that humans are not a hypersensitive species for lethality (376). Additionally, there are differences in the half-life of TCDD in different animals; in rodents, the half-life is 2–4 wks, but in humans, the half-life is approximately 10 yrs, and additional factors influence TCDD pharmacokinetics including the exposure level and the amount of body fat present (377–379). In cell cultures, doses as low as 10^{-11} M are toxic, with decreased viability observed even in cells maintained in nonproliferative states (380).

TCDD binds to the aryl hydrocarbon receptor (AhR), and differences in the affinity for the receptor may be responsible for differences in sensitivity between species (381). The K_d (dissociation constant for receptor-ligand binding kinetics) in human samples typically ranges from 3–15 nM, but in samples from rodents, the K_d is less than 1 nM (382). Importantly, there are also nongenomic pathways affected by TCDD that are mediated by AhR that are typically altered within minutes of TCDD exposure and therefore without changes in transcription (383). Yet many studies suggest that important differences exist between species regarding binding affinity of TCDD for AhR and the toxicity of this chemical, but that other adverse effects, including those related to the endocrine-disrupting activities of TCDD, occur at similar doses (or body burdens) across animal species (384, 385). Thus, it is plausible that AhR affinity alone can predict some, but not all, effects of TCDD and related chemicals.

The mechanisms responsible for many of the endocrine-disrupting activities of TCDD are currently not well understood. Knocking out AhR disrupts morphogenesis of several organ systems even in the absence of a ligand like TCDD, suggesting that this receptor plays important roles in early development (386). AhR is translocated to the nucleus after loss of cell-cell contacts and is often localized to the nucleus in embryonic cells, suggesting that it could have ligand-independent effects on development and/or that endogenous ligands could be present during early development (387). When TCDD is present, AhR translocates to the nucleus and dimerizes with ARNT, the aromatic hydrocarbon receptor nuclear translocator (388). Although the (currently unidentified) physiological activators of AhR are likely to induce rapid on/off signaling via AhR, TCDD and related compounds appear to maintain activation of AhR, and the presence of TCDD prevents the normal action of the AhR signaling pathway in the maintenance of homeostasis (389). This induces changes in the expression of genes and promotes the production of toxic metabolites. These effects may be responsible for some of the endocrine-related endpoints affected by TCDD exposure. Additionally, recent studies have shown complex and intricate interactions between the

AhR and ER signaling pathways (390), suggesting that dioxin may also have indirect effects on some ER-mediated endpoints via AhR signaling.

Teratogenic effects of TCDD have been well documented after high-dose (391, 392) and low-dose exposures (393). These studies show that almost every organ and system in the body is affected by this chemical. High doses that did not produce lethality caused severe weight loss, intestinal hemorrhaging, alopecia, chloracne, edemas, and severe liver damage. Sadly, there are now several examples in humans of accidental exposures after the industrial release of TCDD where a number of individuals have been exposed to large doses (389, 394) as well as a few documented intentional poisonings (395). The tolerated daily intake level was set at 1–4 pg/kg · d, although the doses consumed by nursing infants are likely to exceed these levels by a factor of 10 (375). Adult exposures usually result from the consumption of contaminated foods, and because TCDD is lipophilic, it is concentrated in the fat component of breast milk and therefore passed in large quantities from a nursing mother to her infant.

Using classical toxicology methods, the effects of single TCDD doses were examined in adult male rats, specifically focusing on the effects of this chemical on the number of spermatids per testis and the integrity of the testicular germinal epithelium (396). In one of the earliest studies, Chahoud and colleagues (397) determined a LOAEL of 3 $\mu\text{g}/\text{kg} \cdot \text{d}$ and set the NOAEL at 1 $\mu\text{g}/\text{kg} \cdot \text{d}$ for effects on the testes. Because there are significant differences in the toxicity of TCDD between animal models, and different endpoints have different identified NOAELs, we have selected the 1 $\mu\text{g}/\text{kg} \cdot \text{d}$ identified by Chahoud *et al.* as the cutoff for low-dose studies of this compound. This cutoff is based on the NTP's definition of low dose as occurring at doses lower than those tested in traditional toxicology assessments (2). However, it is important to acknowledge that body burdens that mimic those observed in human populations are likely the best indicators of low doses for TCDD (384), and thus we recommend that future studies determine body burdens after administration of TCDD for the specific strain, origin, and species of animal being tested to ensure that truly low doses, relevant to human populations, are being tested.

Several recent epidemiological studies have indicated that relatively high exposures to TCDD during early life (due to industrial release of high amounts of the chemical) can permanently affect semen quality and sperm count in men (398). Yet epidemiology studies also clearly show that the timing of TCDD exposure can vastly influence the effect of this chemical on spermatogenesis; exposures during perinatal life significantly reduced sperm parameters, but exposures during puberty increased sperm counts; ex-

posures in adulthood had no effect on sperm parameters (399). Thus, it is also important for animal studies to focus on exposures during critical periods for development of the male reproductive tract and spermatogenesis in particular.

We are aware of 18 studies that have examined the effects of low doses ($\leq 1 \mu\text{g}/\text{kg} \cdot \text{d}$) of TCDD during perinatal development on male fertility endpoints in adulthood. The endpoints assessed vary, including epididymal sperm counts, ejaculated sperm number, daily sperm production, sperm transit rate, and percent abnormal sperm, and the sensitivity of these endpoints appears to impact the ability to detect low-dose effects in different studies (400, 401) (Table 5). In total, 16 rodent studies examined the effect of low-dose TCDD on epididymal sperm count; 12 showed significant effects on this endpoint (402–413), whereas the other four did not (414–417). Of the five studies that examined ejaculated sperm counts, four studies (404, 405, 408), including one examining rhesus monkeys (418), showed effects of low-dose TCDD, *i.e.* a significant decrease in sperm counts; one study found no effect (417). Daily sperm production was a less-sensitive endpoint, with four studies showing significant decreases after prenatal exposure to low doses (402, 403, 407, 409) and four studies showing no effects (406, 412, 413, 416); sperm transit rate was examined in only two studies, although both showed significant decreases in sperm transfer rates (403, 410); and finally, three studies determined that low-dose TCDD produced abnormalities in sperm appearance or motility (414, 415, 419), but one study was not able to replicate these findings (417).

When examining the TCDD literature as a whole, the WoE strongly suggests that prenatal exposure to low doses of TCDD affects sperm-related endpoints in adulthood (Table 5). In all, only two studies were unable to detect any effect of TCDD on the sperm endpoints assessed, although both studies found effects of TCDD on other endpoints including the weight of the adult prostate (416) and the timing of puberty (417). No study on TCDD used a positive control, likely due to a paucity of information on the mechanisms of dioxin action, but this raises obvious questions about the ability of these experimental systems to detect effects on spermatogenesis. Finally, some of the inability to detect effects of TCDD could be due to the use of insensitive strains, because 1000-fold differences in sensitivity have been reported for different rodent strains (420).

Even though we have focused the majority of our attention on the effects of low-dose TCDD exposure on spermatogenesis, it should be noted that low doses of this chemical affect a multitude of endpoints in animals, altering immune function (421, 422), indicators of oxidative

TABLE 5. Summary of low-dose animal studies examining the effects of TCDD on spermatogenesis endpoints

Study	Administered dose (time of administration)	Animal	Epididymal sperm count	Ejaculated sperm no.	Daily sperm production	Sperm transit rate	% abnormal sperm
Mably <i>et al.</i> (409)	0.064–1 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased	NA	Decreased	NA	NA
Bjerke and Peterson (402)	1 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased	NA	Decreased	NA	NA
Gray <i>et al.</i> (404)	1 $\mu\text{g}/\text{kg}$ (gestational d 8)	Rat	Not significant	Decreased	NA	NA	NA
	1 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased	Decreased	NA	NA	NA
	1 $\mu\text{g}/\text{kg}$ (gestational d 11)	Hamster	Decreased	Decreased	NA	NA	NA
Sommer <i>et al.</i> (408)	1 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased	Decreased	Decreased	Not significant	Not significant
Wilker <i>et al.</i> (410)	0.5, 1 or 2 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased	NA	Unaffected	Increased	NA
Gray <i>et al.</i> (405)	0.05–1 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased	Decreased	Decreased	NA	NA
Faqi <i>et al.</i> (403)	0.025–0.3 $\mu\text{g}/\text{kg}$ (before mating, then 0.005–0.06 $\mu\text{g}/\text{kg}$ weekly [to dams])	Rat	Decreased	NA	Decreased	Increased	Increased
Loeffler and Peterson (412)	0.25 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased	NA	Unaffected	NA	NA
Ohsako <i>et al.</i> (416)	0.0125–0.8 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Not significant	NA	Unaffected	NA	NA
Ohsako <i>et al.</i> (406)	1 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased	NA	Unaffected	NA	NA
Simanainen <i>et al.</i> (407)	1 $\mu\text{g}/\text{kg}$ /gestational d 18	Rat	Unaffected	NA	Unaffected	NA	NA
	1 $\mu\text{g}/\text{kg}$ /postnatal d 2 (to pups)	Rat	Unaffected	NA	Unaffected	NA	NA
	0.03–1 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased	NA	Decreased	NA	NA
Yonemoto <i>et al.</i> (417)	0.0125–0.8 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Unaffected	Unaffected	NA	NA	Unaffected
Yamano <i>et al.</i> (714)	0.3 or 1 $\mu\text{g}/\text{kg}$ (postnatal d 1 and then every week [to dams])	Rat	Not significant	NA	NA	NA	NA
Ikeda <i>et al.</i> (715)	0.4 $\mu\text{g}/\text{kg}$ (before mating, then 0.08 $\mu\text{g}/\text{kg}$ weekly [to dams])	Rat	Unaffected	NA	NA	NA	NA
Bell <i>et al.</i> (414)	0.05–1 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Increased (at certain ages)	NA	NA	NA	Increased
Bell <i>et al.</i> (415)	0.0024–0.046 $\mu\text{g}/\text{kg}$ (d 12 weeks before pregnancy through parturition)	Rat	Unaffected	NA	NA	NA	Increased
Arima <i>et al.</i> (418)	0.03 or 0.3 $\mu\text{g}/\text{kg}$ (gestational d 20, then 5% of dose monthly [to dams])	Rhesus monkey	Decreased	Not significant	NA	NA	Not significant
Yamano <i>et al.</i> (419)	0.3 or 1 $\mu\text{g}/\text{kg}$ (weekly to dams then pups [all postnatal])	Rat	NA	NA	NA	NA	Increased
Jin <i>et al.</i> (411)	1 $\mu\text{g}/\text{kg} \cdot \text{d}$ (postnatal days 1–4 [to dams])	Mouse	Decreased	NA	NA	NA	NA
Rebourcet <i>et al.</i> (413)	0.01–0.2 $\mu\text{g}/\text{kg}$ (gestational d 15)	Rat	Decreased (at some ages)	NA	Not significant	NA	NA

Not significant indicates trend for effect but did not reach statistical significance. Unaffected means assessed, but no differences were observed relative to controls. Here, low doses were considered any at or below 1 $\mu\text{g}/\text{kg} \cdot \text{d}$ (see text for discussion of how this cutoff was established for rodent studies). NA, Not assessed.

stress (423–425), bone and tooth development (426, 427), female reproduction and timing of puberty (428–430), mammary gland development and susceptibility to cancers (431), behaviors (432, 433), and others. In several cases, lower doses were more effective at altering these endpoints than higher ones (423, 424, 426, 433). Epidemiology studies of nonoccupationally exposed individuals also indicate that serum TCDD levels may be linked to diseases in humans as well (434). Mean serum TCDD levels have decreased by a factor of 7 over a 25-yr period (1972–97) in several industrial nations (435), but results from both animal and epidemiological studies suggest that even the low levels detected now could have adverse effects on health-related endpoints.

G. Perchlorate and thyroid: low-dose effects in humans?

A significant challenge with observing low-dose effects of EDCs in the human population is that human chemical exposures are multivariate along the vectors of time, space, and sensitivities. In addition, chemicals can exert effects on several systems simultaneously. Therefore, associations in human studies between exposures and disease are difficult to reconcile with experimental studies in animal model systems. For this reason, the literature describing the potential impacts of perchlorate contamination on the human population is potentially clarifying because to the best of our knowledge, perchlorate exerts only a single effect, and the pharmacology of perchlorate exposures has been studied in human volunteers (436). This

literature offers a unique perspective into the issue of low-dose effects, perhaps providing important hypotheses to explain mechanistically why high-dose, short-term experiments can fail to predict the outcome of low-dose, lifetime exposures.

In the 2001–2002 NHANES dataset, perchlorate was detected in the urine of each of the 2820 samples tested (437). This widespread exposure means that the human population is being continuously exposed because perchlorate has a half-life in the human body of about 8 h (438). Human exposures to perchlorate are likely attributed to both contaminated drinking water and food (439); in fact, a recent analysis concludes that the majority of human exposure to perchlorate comes from food (440).

The predominant theory proposed to explain the source of perchlorate contamination in the United States is that it has been employed for many decades as the principal oxidant in explosives and solid rocket fuels (441). Perchlorate is chemically stable when wet and persists for long periods in geological systems and in ground water. Because of disposal practices during the 1960s through 1990s, perchlorate became a common contaminant of ground water in the United States (441, 442). Perchlorate is also formed under certain kinds of natural conditions (443), although the relative contributions to human exposure of these different sources is not completely understood. As a result of perchlorate contamination of natural waters, the food supply has become contaminated through irrigation in part because both aquatic and terrestrial plants can concentrate perchlorate more than 100-fold over water levels (444).

This exposure profile in the human population is important because high doses of perchlorate are known to reduce functioning of the thyroid gland, and poor thyroid function is an important cause of developmental deficits and adult disease (445). The primary question is: at what dose does perchlorate inhibit thyroid function sufficiently to cause disease? The current literature, reviewed below, supports the view that background exposure may affect thyroid function in adult women. These exposure levels, however, are considerably lower than predicted by early toxicology experiments in humans.

Perchlorate reduces thyroid function by inhibiting iodide uptake by the sodium/iodide symporter (NIS) (446), which is the only known effect of perchlorate on human physiology (438). NIS is responsible for transporting iodide into the thyroid gland, which is required for the production of thyroid hormone (447). However, NIS is also expressed in the gut (448, 449), in lactating breast (448, 450, 451), and in placenta (452), presumably all as a delivery mechanism for iodide to the developing and adult thyroid gland. Because the NIS transports perchlorate

(450), the pathway by which humans take up and concentrate perchlorate is the same as the pathway by which humans take up and concentrate iodide. Interestingly, NIS expression in the human fetal thyroid gland is the rate-limiting step in production of thyroid hormone (453). Moreover, NIS transport of perchlorate explains why high levels of perchlorate are found in human amniotic fluid (454, 455) and breast milk (456–459).

This effect of perchlorate on thyroid function is important because thyroid hormone is essential for normal brain development, body growth as well as for adult physiology (445, 460). Moreover, it has become clear that even small deficits in circulating thyroid hormone in pregnant women (461, 462) or neonates (463) have permanent adverse outcomes. In fact, recent work indicates that very subtle thyroid hormone insufficiency in pregnant women is associated with cognitive deficits in their children (461). Because of the importance of thyroid hormone in development and adult physiology, and because perchlorate is a potent inhibitor of iodide uptake and thyroid hormone synthesis, identifying the dose at which these events occur is critical.

Perchlorate was used medically to reduce circulating levels of thyroid hormone in patients with an overactive thyroid gland in the 1950s and 1960s (reviewed in Ref. 446); therefore, it was reasonable to examine the dose-response characteristics of perchlorate on the human thyroid gland. Because perchlorate inhibits iodide uptake, several studies were performed to evaluate the effect of perchlorate exposure on iodide uptake inhibition in human volunteers (438, 464–466). In one study, 0.5 or 3 mg/d (approximately 0.007 and 0.04 mg/kg · d) perchlorate was administered to healthy volunteers ($n = 9$ females and 5 males, age 25–65 yr), and no effects were observed (466). Of course, it is important to note that the 2 wk of administration tested in this study is not sufficient to see any effect on serum concentrations of T_4 or TSH; the healthy thyroid can store several months' worth of thyroid hormone in the gland (467). Another small study also found no effects of administering 3 mg/d (approximately 0.04 mg/kg · d) on any thyroid endpoint assessed ($n = 8$ adult males) (464).

In contrast, two studies examining adult volunteers administered perchlorate found effects of this chemical on at least one endpoint. The first found that radioactive iodide uptake was affected by 2 wk of exposure to 10 mg/d (0.13 mg/kg · d), but other measures of thyroid function were not altered ($n = 10$ males) (465). The second examined adults ($n = 37$) given doses ranging from 0.007–0.5 mg/kg · d; all but the lowest dose altered radioactive iodide uptake, and only the highest dose altered TSH levels (438). These studies were interpreted to suggest that adults would have to consume 2 liters of drinking water daily that

was contaminated with at least 200 ppb (200 $\mu\text{g}/\text{liter}$) perchlorate to reach a level in which iodide uptake would begin to be inhibited. Yet, these administered doses are high and relatively acute, so the derivation of a safe dose from these studies, applied to vulnerable populations such as those with low iodide intake, has been strongly disputed (471).

Studies of occupational exposures have also been used to examine the effects of exposure to relatively high levels of perchlorate. In the first such study, more than 130 employees were separated into eight groups based on exposure estimates from airborne perchlorate in the workplace (472). The authors found that individuals with longer daily exposures to perchlorate, due to longer work shifts, had significant decreases in TSH levels compared with individuals with shorter exposures. But this study was hampered because actual exposure levels were not measured via urine or blood samples. A second study examined 37 employees exposed to perchlorate and 21 control employees from an azide factory; actual exposure measures were not conducted, but estimates were calculated based on exposures to perchlorate dust and air samples (473). This study found no effects of perchlorate exposures on any thyroid endpoint, although the sample size examined was small. In the final occupational exposure study, serum perchlorate levels were measured and compared with several measures of thyroid function in workers ($n = 29$) who had spent several years as employees in a perchlorate production plant (474). In this study, the most complete because of the biomonitoring aspect of the exposure measures, higher perchlorate levels were associated with lower radioactive iodide uptake, higher urinary iodide excretion, and higher thyroid hormone concentrations.

Although iodide uptake was often inhibited in these studies, serum thyroid hormones were typically not altered, perhaps because of sufficient stored hormone. Based on these observations, the National Academy Committee to Assess the Health Implications of Perchlorate Ingestion (467) estimated that perchlorate would have to inhibit thyroid iodide uptake by about 75% for several months to cause a reduction in serum thyroid hormones. Moreover, the drinking water concentration of perchlorate required for this kind of inhibition was estimated to be over 1,000 ppb (438). Therefore, the National Academy of Sciences committee recommended a reference dose of 0.0007 $\text{mg}/\text{kg} \cdot \text{d}$ (467), based on the dose at which perchlorate could inhibit iodide uptake, and the EPA used this value to set a provisional drinking water standard of 15 ppb.

Considering these data and general knowledge about the thyroid system, it was unexpected that Blount *et al.*

(475) would identify a positive association between urinary iodide and serum TSH in adult women in the NHANES 2001–2002 dataset. Yet several features of this dataset were consistent with a causal action of perchlorate on thyroid function. First, in the general population of adult women, urinary perchlorate was positively associated with serum TSH. In the population of adult women who also had low urinary iodide, however, urinary perchlorate was more strongly associated with serum TSH and was negatively associated with serum T_4 . The strength of this association was such that the authors calculated that women at the 50th percentile of perchlorate exposure experienced a 1 $\mu\text{g}/\text{dl}$ T_4 reduction (reference range = 5–12 $\mu\text{g}/\text{dl}$). Should this magnitude of reduction in serum T_4 occur in a neonate, measurable cognitive deficits would also be present (476). Finally, Steinmaus *et al.* (477), using the same NHANES dataset, showed that women with low urinary iodide who smoke had an even stronger association between urinary perchlorate and measures of thyroid function. Tobacco smoke delivers thiocyanates, which also inhibit NIS-mediated iodide uptake (446).

The NHANES dataset suggests that perchlorate exposures of 0.2–0.4 $\mu\text{g}/\text{kg} \cdot \text{d}$ (440) are associated with depressed thyroid function, even when urinary iodide is not reduced. This is a considerably lower dose than the 7 $\mu\text{g}/\text{kg} \cdot \text{d}$ dose required to suppress iodide uptake in the Greer *et al.* (438) study or the 500 $\mu\text{g}/\text{kg} \cdot \text{d}$ the NAS estimated would be required for several months to actually cause a decline in serum T_4 . Therefore, it is reasonable to question whether these associations represent a causative relationship between perchlorate and thyroid function.

A number of epidemiological studies have been published to test for a relationship between perchlorate exposure and thyroid function. Early work used neonatal screening data for T_4 as a measure of thyroid function, and the city of birth (Las Vegas, NV, compared with Reno, NV) as a proxy measure of exposure (478, 479). The reported findings were negative, but we now know that all Americans are exposed to perchlorate, so there was considerable misclassification of exposure, and no relationship should have been observed. Several additional studies using similar flawed designs also found no relationship between proxy measures of perchlorate exposures and clinical outcomes (480–484).

A recent study of the neonatal screening data from 1998 in California identified a strong association between neonatal TSH and whether or not the mother resided in a contaminated area (485). This study included over 497,000 TSH measurements and 800 perchlorate measurements. In addition, they used as a cut-off a variety of TSH levels (as opposed to the 99.9th percentile used for the diagnosis of congenital hypothy-

roidism), indicating that perchlorate exposure is not associated with congenital hypothyroidism. Two additional studies have shown similar relationships between perchlorate and TSH levels, particularly in families with a history of thyroid disease (486, 487).

Several studies in pregnant women have failed to identify a relationship between perchlorate exposure and measures of thyroid function (488–490). Although these are important studies that need to be carefully scrutinized, they do not replicate or refute the NHANES dataset. It thus remains important to conduct additional studies exploring the relationship between background exposure to perchlorate and thyroid function in adults, pregnant women, neonates, and infants. This effort will be challenging because of the different characteristics of thyroid function and hormone action at different life stages (460). In addition, it will be important to obtain individual measurements of exposures to perchlorate and other NIS inhibitors (thiocyanate and nitrate), and iodide itself as well as individual measures of thyroid function (free and total T₄ and TSH).

If background levels of perchlorate affect thyroid function in any segment of the population, it will be challenging to explain how the high-dose, short-term experiments of Greer *et al.* (438) completely underestimated the sensitivity of the human thyroid gland to perchlorate exposure. One possibility is that physiological systems respond to short durations of robust stress with compensatory mechanisms that reset during periods of long-term stress.

When these data are examined together, several important issues are raised. First, this example illustrates the difficulties inherent in studying human populations; epidemiology yields associations, not cause-effect relationships, in many cases using surrogate markers for perchlorate, and is not able to distinguish short- *vs.* long-term exposure duration. Second, our WoE analysis suggests that there is weak evidence for low-dose effects of perchlorate; further research is needed. The relationship between low-dose perchlorate exposures and thyroid endpoints would be strengthened by the addition of studies that measure biological concentrations of perchlorate and compare them with thyroid endpoints in neonates and other vulnerable populations. Third, the published studies that reported low-dose effects of perchlorate typically examined very specific populations, with several focusing on women with low iodine intake. This observation suggests that some groups may be more vulnerable to low doses of perchlorate than others (491).

H. Low-dose summary

These examples, and the examples of low-dose effects in less well-studied chemicals (Table 3), provide evidence

that low-dose effects are common in EDC research and may be the default expectation for all chemicals with endocrine activity. Many known EDCs have not been examined for low-dose effects, but we predict that these chemicals will have effects at low doses if studied appropriately. Although studies unable to detect effects at low doses have received attention, including some studies designed to replicate others that reported low-dose effects, the majority of these studies contain at least one major design flaw. Thus, a WoE approach clearly indicates that low-dose effects are present across a wide span of chemical classes and activities.

III. Nonmonotonicity in EDC Studies

A concept related to low dose is that of nonmonotonicity. As noted in *Section I.B*, in a monotonic response, the observed effects may be linear or nonlinear, but the slope does not change sign (Fig. 3, A and B). In contrast, a dose-response curve is nonmonotonic when the slope of the curve changes sign somewhere within the range of doses examined (Fig. 3C). NMDRCs are often U-shaped (with maximal responses of the measured endpoint observed at low and high doses) or inverted U-shaped (with maximal responses observed at intermediate doses) (Fig. 3C, *top panels*). Some cases are more complicated, with multiple points along the curve at which the slope of the curve reverses sign (Fig. 3C, *bottom left*). Nonmonotonicity is not synonymous with low dose, because there are low-dose effects that follow monotonic dose-response curves. Thus, it is not required that a study include doses that span from the true low-dose range to the high toxicological range to detect nonmonotonicity. The consequence of NMDRCs for toxicity testing is that a safe dose determined from high doses does not guarantee safety at lower, untested doses that may be closer to current human exposures.

Examples of NMDRCs from the cell culture, animal, and epidemiological literature will be discussed in detail in *Section III.C*. Importantly, our review of the literature finds that NMDRCs are common in the endocrine and EDC literature. In fact, it is plausible that, considering the mechanisms discussed below, NMDRCs are not the exception but should be expected and perhaps even common.

A. Why is nonmonotonicity important?

NMDRCs in toxicology and in the regulatory process for EDCs are considered controversial. In addition to discussions of whether NMDRCs exist, there is also discussion of whether those that do exist have relevance to

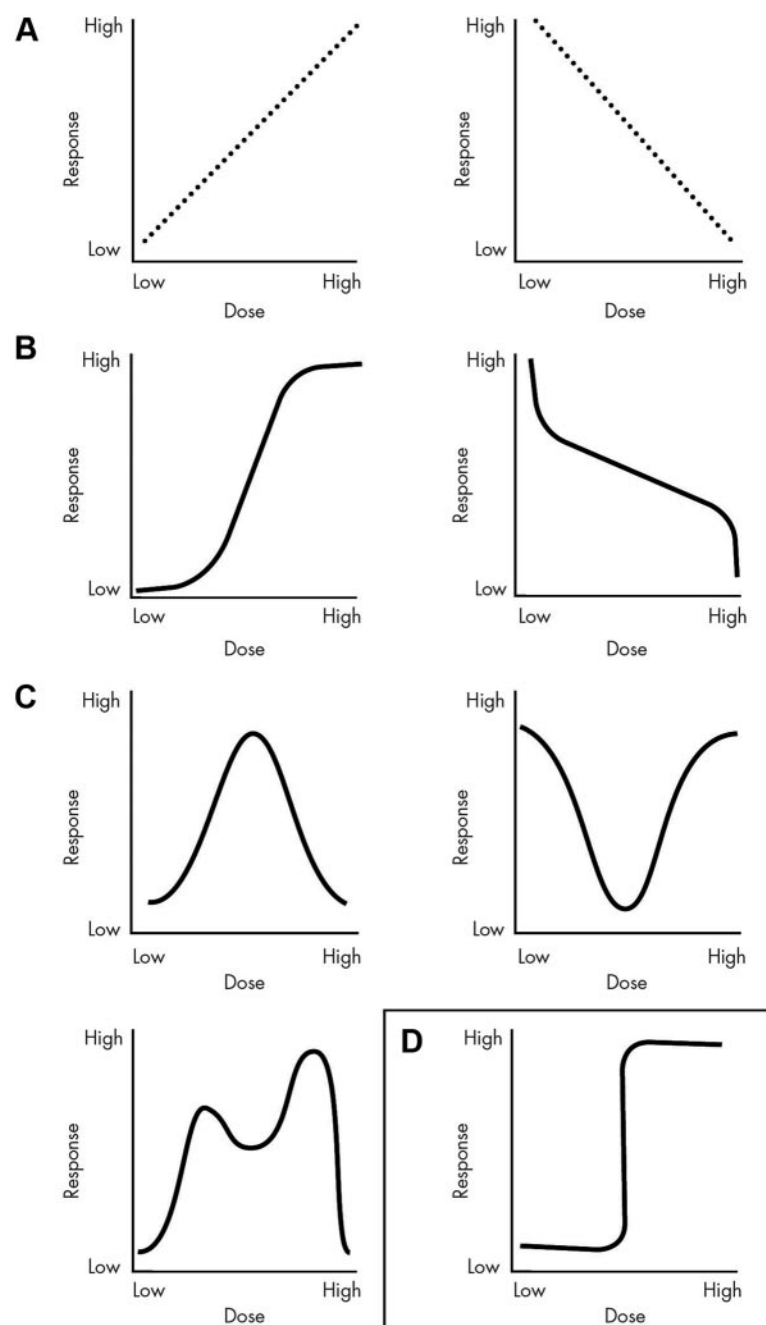
Figure 3.

Figure 3. Examples of dose-response curves. A, Linear responses, whether there are positive or inverse associations between dose and effect, allow for extrapolations from one dose to another. Therefore, knowing the effects of a high dose permits accurate predictions of the effects at low doses. B, Examples of monotonic, nonlinear responses. In these examples, the slope of the curve never changes sign, but it does change in value. Thus, knowing what happens at very high or very low doses is not helpful to predict the effect of exposures at moderate doses. These types of responses often have a linear component within them, and predictions can be made within the linear range, as with other linear responses. C, Displayed are three different types of NMDRCs including an inverted U-shaped curve, a U-shaped curve, and a multiphasic curve. All of these are considered NMDRCs because the slope of the curve changes sign one or more times. It is clear from these curves that knowing the effect of a dose, or multiple doses, does not allow for assumptions to be made about the effects of other doses. D, A binary response is shown, where one range of doses has no effect, and then a threshold is met, and all higher doses have the same effect.

toxicological determination of putative safe exposures. In the standard practice of regulatory toxicology, the calculated safe dose, also called a reference dose, is rarely tested. In a system that is responding nonmonotonically, it is not appropriate to use a high-dose test to predict low-dose effects. Unfortunately, all regulatory testing for the effects of chemical exposures assume that this is possible. All current exposure standards employed by government agencies around the world, including the FDA and EPA, have been developed using an assumption of monotonicity (492, 493). The low-dose range, which presumably is what the general public normally experiences, is rarely, if ever, tested directly.

The standard procedure for regulatory testing typically involves a series of tests to establish the lowest dose at which an effect is observable (the LOAEL), then a dose beneath that at which no effect is observable (the NOAEL). Then a series of calculations are used to acknowledge uncertainty in the data, species differences, age differences, *etc.*, and those calculations, beginning with the LOAEL or the NOAEL, produce a reference dose that is presumed to be a safe exposure for humans (Fig. 4). Typically, the reference dose is 3- to 1000-fold lower than the NOAEL. That reference dose then becomes the allowable exposure and is deemed safe, even when it is never examined directly. For chemicals with monotonic linear dose-response curves (Fig. 3A), this may be appropriate. But for any chemicals that display nonmonotonic patterns, it is likely to lead to false negatives, *i.e.* concluding that exposure to the reference dose is safe when in fact it is not.

As described above, there are other nonlinear dose-response curves that are monotonic (Fig. 3B). These curves may also present problems for extrapolating from high doses to low doses because there is no linear relationship that can be used to predict the effects of low doses. Equally troubling for regulatory purposes are responses that have a binary response rather than a classical dose-response curve (Fig. 3D). In these types of responses, one range of doses has no effect on an endpoint, and then a threshold is met, and all higher doses have the same effect. An example is seen in the atrazine literature, where doses below 1 ppb had no effect on the size of the male larynx but doses

at or above 1 ppb produced a significant decrease in size of approximately 10–15% (336). Even doses of 200 ppb, the toxicological NOEL, produce the same effect. Thus, this all-or-none effect is observed because atrazine does not shrink the larynx; instead, it removes the stimulatory agent (*i.e.* androgens). In the absence of some threshold dose of androgen, the larynx simply remains at the unstimulated (female) size. The EPA's assessment of this study and others was that the lack of a dose-dependent response negates the importance of this effect (352). The lack of a dose response for a threshold effect like larynx size does not mean that the effects are not dose dependent; thus, understanding these types of effects and their implications for risk assessments is essential for determining the safe levels of chemicals.

It is important to mention here that the appropriateness of determining NOAEL concentrations, and therefore calculating reference doses, from exposures to endogenous hormones or EDCs has been challenged by several studies (Fig. 4A) (494–496). These studies show that hormonally active agents may still induce significant biological effects even at extremely low concentrations and that presently available analytical methods or technologies might be unable to detect relatively small magnitudes of effects. Previous discussions of this topic have shown that as the dose gets lower (and approaches zero) and the effect size decreases, the number of animals needed to achieve the power to detect a significant effect would have to increase substantially (497). Even more importantly, the assumption of a threshold does not take into account situations where an endogenous hormone is already above the dose that causes detectable effects and that an exogenous chemical (whether an agonist or antagonist) will modulate the effect of the endogenous hormone at any dose above zero (Fig. 4B). There can thus be no threshold or safe dose for an exogenous chemical in this situation. Forced identification of NOAEL or threshold doses based on the assumption that dose-response curves are always monotonic without considering the background activity of endogenous hormones and the limitations of analytical techniques supports the misconception that hormonally active agents do not have any significant biological effects at low doses. Thus, the concept that a toxic agent has a safe dose that can be readily estimated from the NOAEL derived from testing high, acutely toxic doses is overly simplistic and contradicted by data when applied to EDC (5, 497, 498).

B. Mechanisms for NMDRCs

Previously, the lack of mechanisms to explain the appearance of NMDRCs was used as a rationale for ignoring these phenomena (492, 493). This is no longer acceptable

because there are several mechanisms that have been identified and studied that demonstrate how hormones and EDCs produce nonmonotonic responses in cells, tissues, and animals. These mechanisms include cytotoxicity, cell- and tissue-specific receptors and cofactors, receptor selectivity, receptor down-regulation and desensitization, receptor competition, and endocrine negative ^{FEEDBACK} loops. These mechanisms are well understood, and by providing detailed biological insights at the molecular level into the etiology of NMDRCs, they strongly negate the presumption that has been central to regulatory toxicology that dose-response curves are by default monotonic.

1. Cytotoxicity

The simplest mechanism for NMDRCs derives from the observation that hormones can be acutely toxic at high doses yet alter biological endpoints at low, physiologically relevant doses. Experiments working at concentrations that are cytotoxic are incapable of detecting responses that are mediated by ligand-binding interactions. For example, the MCF7 breast cancer cell line proliferates in response to estradiol in the low-dose range (10^{-12} to 10^{-11} M) and in the pharmacological and toxicological range (10^{-11} to 10^{-6} M), but toxic responses are observed at higher doses (38). Thus, when total cell number is graphed, it displays an inverted U-shaped response to estrogen. But cells that do not contain ER, and therefore cannot be affected by the hormonal action of estradiol, also display cytotoxic responses when treated with high doses of hormone. These results clearly indicate that the effects of estradiol at high doses are toxic via non-ER-mediated mechanisms.

2. Cell- and tissue-specific receptors and cofactors

Some NMDRCs are generated by the combination of two or more monotonic responses that overlap, affecting a common endpoint in opposite ways via different pathways. For example, *in vitro* cultured prostate cell lines demonstrate a nonmonotonic response to increasing doses of androgen where low doses increase cell number and higher doses decrease cell number, thus producing an inverted U-shaped curve (499, 500). Although the parental cell expressed an inverted U-shaped dose-response curve, after a long period of inhibition, the effects on cell number could be segregated by selecting two populations of cells: one that proliferated in the absence of androgens and other cells that proliferated in the presence of high androgen levels (501). Thus, the observed inverted U-shaped response is due to actions via two independent pathways that can be separated from each other in an experimental setting (502). Similarly, estrogens have been shown to induce cell proliferation and inhibit apoptosis in several cell populations, but inhibit proliferation and induce apopto-

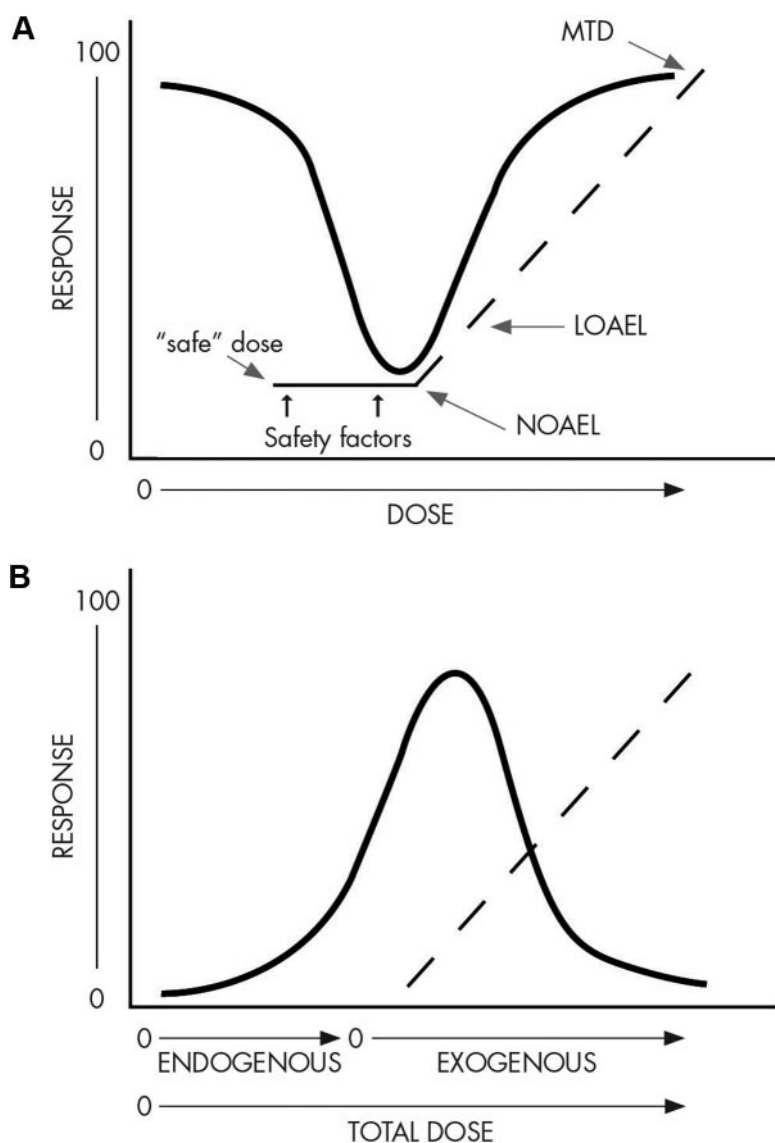
Figure 4.

Figure 4. NOAEL, LOAEL, and calculation of a safe reference dose. A, In traditional toxicology testing, high doses are tested to obtain the maximum tolerated dose (MTD), the LOAEL, and the NOAEL. Several safety factors are then applied to derive the reference dose, *i.e.* the dose at which exposures are presumed safe. This reference dose is rarely tested directly. Yet when chemicals or hormones produce NMDRCs, adverse effects may be observed at or below the reference dose. Here, the doses that would be tested are shown by a dotted line, and the calculated safe dose is indicated by a thick solid line. The actual response, an inverted U-shaped NMDRC, is shown by a thin solid line. B, Experimental data indicate that EDCs and hormones do not have NOAELs or threshold doses, and therefore no dose can ever be considered safe. This is because an exogenous hormone (or EDC) could have a linear response in the tested range (dotted line), but because endogenous hormones are present (thin solid line), the effects of the exogenous hormone are always observed in the context of a hormone-containing system.

sis in others (503, 504), with the combined effect being an inverted U-shaped curve for cell number (505).

Why does one single cell type have different responses to different doses of the same hormone? The case of the prostate cell line described above is reminiscent of the re-

sults described from the transcriptome of MCF7 cells, whereby a discrete global response like cell proliferation manifests at significantly lower estrogen doses than the induction of a single marker gene (135). That a response like cell proliferation requires a significantly lower dose of hormone than the dose needed to induce a given target gene is counterintuitive but factual; it may be interpreted as consistent with the notion that metazoan cells, like cells in unicellular organisms, are intrinsically poised to divide (503, 506, 507) and that quiescence is an induced state (508, 509). The biochemical details underlying these different responses are largely unknown; however, recent studies showed that steroid receptors control only a portion of their target genes directly via promoter binding. The majority of the changes are indirect, through chromatin rearrangements (510, 511).

Why do different cell types (*in vitro* and *in vivo*) have different responses to the same hormone? One answer is that they may express different receptors, and these receptors have different responses to the same hormone. For example, some tissues express only one of the two major ER (ER α and ER β), and actions via these receptors are important not just for responsiveness to hormone but also for cellular differentiation and cross talk between tissue compartments (512). Yet other tissues express both ER α and ER β , and the effects of signaling via these two receptors often oppose each other; *i.e.* estrogen action via ER α induces proliferation in the uterus, but ER β induces apoptosis (154). Complicating the situation further, different responses to a hormone can also be obtained due to the presence of different cofactors in different cell and tissue types (513, 514); these coregulators influence which genes are transcriptionally activated or repressed in response to the presence of hormone. They can also influence ligand selectivity of the receptor and DNA-binding capacity, having tremendous impact on the ability of a hormone to have effects in different cell types (105, 515, 516).

Although much of these activities occur on a biochemical level, *i.e.* at the receptor, there is also evidence that nonmonotonicity can originate at the level of tissue organization. The mammary gland has been used as a model to study inter- and intracompartmental effects of hormone treatment: within the ductal epithelium, estro-

gen has distinct effects during puberty, both inducing proliferation, which causes growth of the ductal tree, and inducing apoptosis, which is required for lumen formation (517, 518); in cell culture, the presence of stromal cells can also enhance the effects of estrogen on epithelial cells (519, 520), suggesting that stromal-epithelial compartmental interactions can mediate the effects of estrogen.

3. Receptor selectivity

NMDRCs can occur because of differences in receptor affinity, and thus the selectivity of the response, at low *vs.* high doses. For example, at low doses, BPA almost exclusively binds to the ER (including mER), but at high doses it can also bind weakly to other hormone receptors, like androgen receptor and thyroid hormone receptor (249, 521). This type of receptor nonselectivity is quite common for EDCs, and it has been proposed that binding to different receptors may be an explanation for the diverse patterns of disease observed after EDC exposures (522). In fact, several of the chemicals shown to have low-dose effects are known to act via multiple receptors and pathways (Table 3). Thus, the effects seen at high doses can be due to action via the binding of multiple receptors, compared with the effects of low doses, which may be caused by action via only a single receptor or receptor family.

4. Receptor down-regulation and desensitization

When hormones bind to nuclear receptors, the ultimate outcome is a change in the transcription of target genes. When the receptor is bound by ligand, an increase in response is observed; as discussed previously in this review, the relationship between hormone concentration and the number of bound receptors, as well as the relationship between the number of bound receptors and the biological effect, is nonlinear (38). After the nuclear receptor is bound by hormone and transcription of target genes has occurred (either due to binding of the receptor at a DNA response element or the relief of a repressive event on the DNA), the reaction eventually must cease; *i.e.* the bound receptor must eventually be inactivated in some way. Thus, nuclear hormone receptors are ubiquitinated and degraded, usually via the proteasome (523). Importantly, the role of the hormone in receptor degradation differs depending on the hormone; binding of estrogen, progesterone, and glucocorticoid mediates the degradation of their receptors (524–526), whereas the presence of hormone may actually stabilize some receptors and prevent degradation (527), and other receptors are degraded without ligand (528). As hormone levels rise, the number of receptors being inactivated and degraded also rises, and eventually the number of receptors being produced cannot maintain the pace of this degradation pathway (523). Fur-

thermore, the internalization and degradation of receptors can also influence receptor production, leading to an even stronger down-regulation of receptor (529). In the animal, the role of receptor down-regulation is actually quite complex, because signaling from one hormone receptor can influence protein levels of another receptor; *i.e.* ER signaling can promote degradation of the glucocorticoid receptor by increasing the expression of enzymes in the proteasome pathway that degrade it (530).

There is also the issue of receptor desensitization, a process whereby a decrease in response to a hormone is not due to a decrease in the number of available receptors but instead due to the biochemical inactivation of a receptor (531). Desensitization typically occurs when repeated or continuous exposure to ligand occurs. Normally seen with membrane-bound G protein-coupled receptors, the activation of a receptor due to ligand binding is quickly followed by the uncoupling of the activated receptor from its G proteins due to phosphorylation of these binding partners (532). Receptor desensitization has been observed for a range of hormones including glucagon, FSH, human chorionic gonadotropin, and prostaglandins (533). Importantly, desensitization and down-regulation can occur in the same cells for the same receptor (534), and therefore, both can play a role in the production of NMDRCs.

5. Receptor competition

Mathematical modeling studies suggest that the mixture of endogenous hormones and EDCs establishes a natural environment to foster NMDRCs. Using mathematical models, Kohn and Melnick (42) proposed that when EDC exposures occur in the presence of endogenous hormone and unoccupied hormone receptors, some unoccupied receptors become bound with the EDC, leading to an increase in biological response (*i.e.* increased expression of a responsive gene, increased weight of an organ, *etc.*). At low concentrations, both the endogenous hormone and the EDC bind to receptors and activate this response, but at high doses, the EDC can outcompete the natural ligand. The model predicts that inverted U-shaped curves would occur regardless of the binding affinity of the EDC for the receptor and would be abolished only if the concentration of natural hormone were raised such that all receptors were bound.

6. Endocrine negative ^{FEEDBACK} loops

In several cases, the control of hormone synthesis is regulated by a series of positive- and negative feedback loops. Several hormones are known to control or influence their own secretion using these feedback systems. In one example, levels of insulin are known to regulate glucose uptake by cells. Blood glucose levels stimulate insulin pro-

duction, and as insulin removes glucose from circulation, insulin levels decline. Thus, NMDRCs can occur as the free/available ligand and receptor concentrations are influenced by one another. In another example, thyroid hormone secretion is stimulated by TSH, and thyroid hormone suppresses TSH; thus, feedback between these two hormones allows thyroid hormone to be maintained in a narrow dose range.

Several studies indicate that these negative feedback loops could produce NMDRCs when the duration of hormone administration is changed (535). For example, short exposures of estrogen induce proliferation in the uterus and pituitary, but longer hormone regimens inhibit cell proliferation (236, 536). Thus, the outcome is one where exposure to a single hormone concentration stimulates an endpoint until negative feedback loops are induced and stimulation ends (537).

7. Other downstream mechanisms

Removing the variability that can come from examining different cell types, or even single cell types in the context of a tissue, studies of cultured cells indicate that different gene profiles are affected by low doses of hormone compared with higher doses. In a study of the genes affected by low *vs.* higher doses of estrogen, researchers found that there were a small number of genes in MCF7 breast cancer cells with very high sensitivity to low doses of estradiol (10 pM) compared with the total number of genes that were affected by higher (30 or 100 pM) exposures (538). But the surprising finding was the pattern of estradiol-induced *vs.* estradiol-suppressed gene expression at high and low doses; when 10 pM was administered, the number of estradiol-suppressible genes was approximately three times higher than the number of estradiol-inducible genes. However, the overall profile of the number of estradiol-suppressible genes was approximately half the total number of estradiol-inducible genes. This observation suggests that low doses of estrogen selectively target a small subset of the total number of estrogen-sensitive genes and that the genes affected by low doses are most likely to be suppressed by that treatment. The mechanisms describing how low doses of estrogen differently affect the expression of genes compared with higher doses have yet to be elucidated, but low doses of estradiol inhibit expression of apoptotic genes (539), indicating that which genes are affected by hormone exposure is relevant to understand how low doses influence cellular activities.

C. Examples of nonmonotonicity

1. Examples of NMDRCs from cell culture

A tremendous amount of theoretical and mathematical modeling has been conducted to understand the produc-

tion of nonlinear and nonmonotonic responses (42, 540). These studies and others suggest that the total number of theoretical response curves is infinite. Yet this does not mean that the occurrence of NMDRCs is speculative; these types of responses are reported for a wide variety of chemicals. Cell culture experiments alone provide hundreds of examples of nonmonotonic responses (see Table 6 for examples). In the natural hormone category, many different hormones produce NMDRCs; this is clearly not a phenomenon that is solely attributable to estrogen and androgen, the hormones that have been afforded the most attention in the dose-response literature. Instead, NMDRCs are observed after cells are treated with a range of hormones, suggesting that this is a fundamental and general feature of hormones.

Chemicals from a large number of categories with variable effects on the endocrine system also produce NMDRCs in cultured cells. These chemicals range from components of plastics to pesticides to industrial chemicals and even heavy metals. The mechanisms for nonmonotonicity discussed in *Section III.B* are likely explanations for the NMDRCs reported in a range of cell types after exposure to hormones and EDCs. Table 6 provides only a small number of examples from the literature, and it should be noted that because these are studies of cells in culture, most of these studies typically examined only a few types of outcomes: cell number (which could capture the effects of a chemical on cell proliferation, apoptosis, or both), stimulation or release of another hormone, and regulation of target protein function, often examined by measuring the phosphorylation status of a target.

2. Examples of NMDRCs in animal studies

Some scientists suggest that nonmonotonicity is an artifact of cell culture, however, a large number of NMDRCs have been observed in animals after administration of natural hormones and EDCs, refuting the hypothesis that this is a cell-based phenomenon only. Similar to what has been observed in cultured cells, the NMDRCs observed in animals also span a large range of chemicals, model organisms, and affected endpoints (Table 7). These results underscore the biological importance of the mechanisms of nonmonotonicity that have been largely worked out *in vitro*.

Although NMDRCs attributable to estrogen treatment are well documented, the induction of NMDRCs is again observed to be a general feature of hormone treatment; a wide range of hormones produce these types of responses in exposed animals. Importantly, a number of pharmaceutical compounds with hormone-mimicking or endocrine-disrupting activities also produce NMDRCs. Finally, as expected from the results of cell culture

TABLE 6. Examples of NMDRCs in cell culture experiments

Chemicals by chemical class	Nonmonotonic effect	Cell type	Refs.
Natural hormones			
17 β -Estradiol	Cell number	MCF7 breast cancer cells	135, 716
	Dopamine uptake	Fetal hypothalamic cells (primary)	717
	pERK levels, prolactin release	GH3/B6/F10 pituitary cells	41, 718, 719
	β -Hexosaminidase release	HMC-1 mast cells	720
	Cell number	Vascular smooth muscle cells	721
	Production of L-PGDS, a sleep-promoting substance	U251 glioma cells	722
5 α -Dihydrotestosterone	Cell number	LNCaP-FGC prostate cancer cells	499
	Cell number, kinase activity	Vascular smooth muscle cells	721
5 α -Androstenedione	Cell number	LNCaP-FGC prostate cancer cells	499
Corticosterone	Mitochondrial oxidation, calcium flux	Cortical neurons (primary)	723
Insulin	Markers of apoptosis (in absence of glucose)	Pancreatic β -cells (primary)	724
Progesterone	Cell number	LNCaP-FGC prostate cancer cells	499
Prolactin	Testosterone release	Adult rat testicular cells (primary)	725
hCG	Testosterone release	Adult rat testicular cells (primary)	725
T ₃	Rate of protein phosphorylation	Cerebral cortex cells (primary, synaptosomes)	726
	<i>LPL</i> mRNA expression	White adipocytes (rat primary)	727
GH	<i>IGF-I</i> expression	Hepatocytes (primary cultures from silver sea bream)	728
Pharmaceutical hormones			
DES	Cell number	MCF7 breast cancer cells	716
	Prolactin release	GH3/B6/F10 pituitary cells	41
Ethinyl estradiol	CXCL12 secretion	MCF7 breast cancer cells, T47D breast cancer cells	729
R1881 (synthetic androgen)	Cell number	LNCaP-FGC cells	499
Trenbolone	Induction of micronuclei	RTL-W1 fish liver cells	730
Plastics			
BPA	Cell number	MCF7 breast cancer cells	135, 716
	Dopamine efflux	PC12 rat tumor cells	40
	pERK levels, intracellular Ca ²⁺ changes, prolactin release	GH3/B6/F10 pituitary cells	41, 718
	Cell number	LNCaP prostate cancer cells	731
DEHP	Number of colonies	<i>Escherichia coli</i> and <i>B. subtilis</i> bacteria	732
Di- <i>n</i> -octyl phthalate	Number of colonies	<i>E. coli</i> and <i>B. subtilis</i> bacteria	732
Detergents, surfactants			
Octylphenol	Cell number	MCF7 breast cancer cells	716
	Dopamine uptake	Fetal hypothalamic cells (primary)	717
	pERK levels	GH3/B6/F10 pituitary cells	718
	hCG-stimulated testosterone levels	Leydig cells (primary)	733
Propylphenol	pERK levels	GH3/B6/F10 pituitary cells	718
Nonylphenol	pERK levels, prolactin release	GH3/B6/F10 pituitary cells	41, 718
	β -Hexosaminidase release	HMC-1 mast cells	720
	Cell number	MCF7 breast cancer cells	135
PAH			
Phenanthrene	All-trans retinoic acid activity	P19 embryonic carcinoma cells	734, 735
Benz(a)acridine	All-trans retinoic acid activity	P19 embryonic carcinoma cells	734
Naphthalene	hCG-stimulated testosterone	Pieces of goldfish testes	736
B-naphthoflavone	hCG-stimulated testosterone	Pieces of goldfish testes	736
Retene	hCG-stimulated testosterone	Pieces of goldfish testes	736
Heavy metals			
Lead	Estrogen, testosterone, and cortisol levels	Postvitellogenic follicles (isolated from catfish)	737
Cadmium	Expression of angiogenesis genes	Human endometrial endothelial cells	738

(Continued)

TABLE 6. Continued

Chemicals by chemical class	Nonmonotonic effect	Cell type	Refs.
Phytoestrogens and natural antioxidants			
Genistein	Cell number	Caco-2BBE colon adenocarcinoma cells	739
	CXCL12 secretion, cell number	T47D breast cancer cells	729
	Cell number, cell invasion, MMP-9 activity	PC3 prostate cancer cells	740
	pJNK levels, Ca ²⁺ flux	GH3/B6/F10 pituitary cells	719
Coumesterol	Prolactin release, pERK levels	GH3/B6/F10 pituitary cells	719
Daidzein	Prolactin release, pERK levels	GH3/B6/F10 pituitary cells	719
	Cell number	MCF7 breast cancer cells	135
	Cell number	LoVo colon cancer cells	741
Resveratrol	Expression of angiogenesis genes	Human umbilical vein endothelial cells	742
Trans-resveratrol	pERK levels, Ca ²⁺ flux	GH3/B6/F10 pituitary cells	719
Artelastochromene	Cell number	MCF7 breast cancer cells	743
Carpelastofuran	Cell number	MCF7 breast cancer cells	743
Biochanin A	Induction of estrogen-sensitive genes in the presence of testosterone	MCF7 breast cancer cells	744
Licoflavone C	Induction of estrogen-sensitive genes	Yeast bioassay	745
Quercetin	Aromatase activity	H295R adrenocortical carcinoma cells	746
	Cell number	SCC-25 oral squamous carcinoma cells	747
Dioxin			
TCDD	Cell number, gene expression	M13SV1 breast cells	748
PCB			
PCB-74	Cell viability, GnRH peptide levels	GT1-7 hypothalamic cells	749
PCB-118	Cell viability, GnRH peptide levels	GT1-7 hypothalamic cells	749
Aroclor 1242 (PCB mixture)	β -Hexosaminidase release	HMC-1 mast cells	720
POP mixture	Apoptosis of cumulus cells	Oocyte-cumulus complexes (primary, isolated from pigs)	750
Herbicides			
Glyphosate-based herbicide (Round-Up)	Cell death, aromatase activity, ER β activity	HepG2 liver cells	751
Atrazine	Cell number	IEC-6 intestinal cells	752
Insecticides			
Endosulfan	Cell number	IEC-6 intestinal cells	752
	β -Hexosaminidase release	HMC-1 mast cells	720
	ATPase activity of P-glycoprotein	CHO cell extracts	753
Diazinon	Cell number	IEC-6 intestinal cells	752
Dieldrin	β -Hexosaminidase release	HMC-1 mast cells	720
DDT	Cell number	MCF7 breast cancer cells	144
DDE	β -Hexosaminidase release	HMC-1 mast cells	720
	Prolactin release	GH3/B6/F10 pituitary cells	41
3-Methylsulfonyl-DDE	Cortisol and aldosterone release, expression of steroidogenic genes	H295R adrenocortical carcinoma cells	754
Fungicides			
Hexachlorobenzene	Transcriptional activity in the presence of DHT	PC3 prostate cancer cells	755
Prochloraz	Aldosterone, progesterone, and corticosterone levels; expression of steroidogenic genes	H295R adrenocortical cells	756
Ketoconazole	Aldosterone secretion	H295R adrenocortical cells	757
Fungicide mixtures	Aldosterone secretion	H295R adrenocortical cells	757
PBDE			
PBDE-49	Activation of ryanodine receptor 1	HEK293 cell (membranes)	758
PBDE-99	Expression of <i>GAP43</i>	Cerebral cortex cells (primary)	759

Due to space concerns, we have not elaborated on the shape of the curve (U, inverted U, or other nonmonotonic shape) or the magnitude of observed effects in this table. CXCL12, Chemokine (C-X-C motif) ligand 12; DEHP, bis(2-ethylhexyl) phthalate; DHT, dihydrotestosterone; hCG, human chorionic gonadotropin; MMP, matrix metalloproteinase; PAH, polyaromatic hydrocarbons; PBDE, polybrominated diphenyl ethers; PCB, polychlorinated biphenyl; pERK, phospho-ERK; PGDS, prostaglandin-D synthase; pJNK, phospho-c-Jun N-terminal kinase.

TABLE 7. Examples of NMDRCs in animal studies

Chemicals by chemical class	Nonmonotonic effect	Organ/sex/animal	Refs.
Natural hormones			
17 β -Estradiol	Morphological parameters	Mammary gland/female/mice	138, 541
	Accumulation of cAMP	Pineal/female/rats	760
	Prostate weight	male/mice	689
	Uterine weight	female/mice	761
	Antidepressant effects, measured by immobility assay	Behavior/male/mice	762
	Nocturnal activity, gene expression in preoptic area	Brain and behavior/female/mice	763
Corticosterone	Spatial memory errors	Behavior/male/rats	764
	Cholinergic fiber loss in cortex after treatment with neurodegenerative drugs	Brain/male/rats	765
	Mitochondrial metabolism	Muscle/male/rats: strain differences	766
	Contextual fear conditioning	Behavior/male/rats	767
	Locomotor activity	Behavior/male/captive Adelie penguins	768
Glucocorticoid	Na ⁺ /K ⁺ -ATPase activity	Brain/tilapia (fish)	769
Testosterone	Na ⁺ /K ⁺ -ATPase activity	Brain/tilapia (fish)	769
	Gonadotropin subunit gene expression	Pituitary/sexually immature goldfish	770
11 β -Hydroxyandrosterone	Gonadotropin subunit gene expression	Pituitary/sexually immature goldfish	770
T ₄	Bone growth	Tibia/male/rats with induced hypothyroidism	771
Leptin	Insulin production (in the presence of glucose)	Pancreas/male/rats	560
Oxytocin	Infarct size, plasma LDH levels, creatine kinase activity after ischemia/ reperfusion injury	Brain and blood/male/rats	772
	Memory retention	Behavior/male/mice	773
Melatonin	Brain infarction and surviving neuron number after injury	Brain/female/rats	774
Dopamine	Memory	Brain/both/rhesus monkey	775
	Neuronal firing rate	Brain/male/rhesus monkey	776
Pharmaceutical			
DES	Sex ratio, neonatal body weight, other neonatal development	Mice	777
	Adult prostate weight	Male/mice	689
	Uterine weight	Female/mice	761
	Expression of PDGF receptor	Testes/male/rats	778
	Morphological parameters	Mammary gland/male and female/mice	779
Estradiol benzoate	Dorsal prostate weight, body weight	Male/rats	780
	Sexual behaviors, testes morphology	Male/zebra finches (birds)	781
Ethinyl estradiol	GnRH neurons	Brain/zebrafish	782
Tamoxifen	Uterine weight	Female/mice	761
Fluoxetine (antidepressant)	Embryo number	<i>Potamopyrgus antipodarum</i> (snails)	783
Fadrozole (aromatase inhibitor)	Aromatase activity	Ovary/female/fathead minnows	784
Plastics			
BPA	Fertility	Reproductive axis /female/mice	316
	Reproductive behaviors	Behavior/male/rats	785
	Protein expression	Hepatopancreas/male/ <i>Porcellio scaber</i> (isopod)	786
	Timing of vaginal opening, tissue organization of uterus	Reproductive axis/female/mice	577
	Expression of receptors in embryos	Brain and gonad/both/ mice	787
DEHP	Aromatase activity	Hypothalamus/male/rats	788
	Cholesterol levels	Serum/male/rats	569
	Timing of puberty	Reproductive axis /male/rats	789
	Body weight at birth, vaginal opening, and first estrous	Female/rats	790
	Seminal vesicle weight, epididymal weight, testicular expression of steroidogenesis genes	Male/rats	791
	Responses to allergens, chemokine expression	Skin/male/mice	792

(Continued)

TABLE 7. Continued

Chemicals by chemical class	Nonmonotonic effect	Organ/sex/animal	Refs.
Detergents, surfactants			
Nonylphenol ethoxylate	Fecundity	<i>Biomphalaria tenagophila</i> (snails)	793
Octylphenol	Embryo production	<i>P. antipodarum</i> (snails)	794
	Spawning mass and egg numbers	<i>Marisa cornuarietis</i> (snails)	795
Semicarbazide	Timing of preputial separation, serum DHT	Male/rats	796
Antimicrobial			
Triclocarban	Fecundity	<i>P. antipodarum</i> (snails)	797
PCB			
Mixture of PCB	Corticosterone levels	Male/kestrels (birds)	798
Environmental PCB mixture	Corticosterone levels	Female/tree swallows (birds)	799
UV filters			
Octyl methoxycinnamate	Activity, memory	Behavior/both/rats	800
Aromatic hydrocarbons			
B-naphthoflavone	Testosterone	Plasma/male/goldfish	736
Toluene	Locomotor activity	Behavior/male/rats	801
Dioxins			
TCDD	Cell-mediated immunity	Immune system/male/ rats	802
	Proliferation after treatment with chemical carcinogen	Liver/female/rats	803
Heavy metals			
Cadmium	Expression of metallothionein, <i>pS2/TFF1</i>	Intestine and kidney/ female/rats	804
	Activity of antioxidant enzymes	Earthworms	805
	Size parameters, metamorphic parameters	<i>Xenopus laevis</i>	806
Lead	Growth, gene expression	<i>Vicia faba</i> seedlings (plant)	807
	Retinal neurogenesis	Eye and brain/female/rats	808
Selenium	DNA damage, apoptotic index	Prostate/male/dogs	809
	Hatching failure	Eggs/red-winged blackbirds (wild population)	810
Phytoestrogens			
Genistein	Aggressive, defensive behaviors	Behavior/male/mice	811
	Retention of cancellous bone after ovariectomy	Tibia bones/female/rat	812
	Expression of <i>OPN</i> , activation of Akt	Prostate/male/mice	740
Resveratrol	Angiogenesis	Chorioallantoic membrane/chicken embryos	742
	Ulcer index after chemical treatment, expression of gastroprotective genes	Stomach/male/mice	813
Phytochemicals			
Phlorizin	Memory retention	Behavior/male/mice	814
Herbicides			
Atrazine	Time to metamorphosis	Thyroid axis/ <i>Rhinella arenarum</i> (South American toad)	815
	Survivorship patterns	Four species of frogs	363
	Growth parameters	<i>Bufo americanus</i>	816
Pendimethalin	Expression of <i>AR</i> , <i>IGF-I</i>	Uterus/female/mice	817
Commercial mixture with mecoprop, 2,4-dichlorophenoxyacetic acid and dicamba	Number of implantation sites, number of live births	Female/mice	818
Simazine	Estrous cyclicity	Reproductive axis/female/rat	819
Insecticides			
Permethrin	Dopamine transport	Brain/male/mice	820
Heptachlor	Dopamine transport	Brain/male/mice	820
DDT	Number of pups, sex ratios, neonatal body weight, male anogenital distance	Mice	777
Methoxychlor	Number of pups, anogenital distance (males and females), neurobehaviors (males and females)	Mice	777
Chlorpyrifos	Body weight	Male/rats	821
	Antioxidant enzyme activity	<i>Oxya chinensis</i> (locusts)	822
Malathion	Antioxidant enzyme activity	<i>O. chinensis</i> (locusts)	822

(Continued)

TABLE 7. Continued

Chemicals by chemical class	Nonmonotonic effect	Organ/sex/animal	Refs.
Fungicides			
Carbendazim	Liver enzymes, hematology parameters	Blood and liver/male/rats	823
Chlorothalonil	Survival, immune response, corticosterone levels	Several amphibian species	686
Vinclozolin	Protein expression	Testes/male/ <i>P. scaber</i> (isopod)	786

Due to space concerns, we have not elaborated on the shape of the curve (U, inverted U, or other nonmonotonic shape) or the magnitude of observed effects in this table. DEHP, Bis(2-ethylhexyl) phthalate; DHT, dihydrotestosterone; LDH, lactate dehydrogenase; PCB, polychlorinated biphenyl; PDGF, platelet-derived growth factor.

experiments, chemicals with many different modes of action generate NMDRCs in treated animals.

Perhaps most striking is the range of endpoints affected, from higher-order events such as the number of viable offspring (which could be due to alterations in the reproductive tissues themselves or the reproductive axis), to behavioral effects, to altered organ weights, and to lower-order events such as gene expression. The mechanisms responsible for these nonmonotonic phenomena may be similar to those studied in cell culture systems, although

additional mechanisms are likely to be operating *in vivo* such as alterations in tissue organization (541) and the interactions of various players in the positive and negative feedback loops of the endocrine system.

3. Examples of NMDRCs in the epidemiology literature

Perhaps not surprisingly, natural hormones produce NMDRCs in human populations as well (Table 8). Although the methods needed to detect NMDRCs in humans are specific to the field of epidemiology, these results sup-

TABLE 8. NMDRCs for natural hormones identified in the epidemiology literature

Hormone	Affected endpoint	NMDRC	Study subjects	Refs.
Testosterone (free)	Incidence of coronary events	Incidence of 25% at extremes of exposure, 16% at moderate exposure	Rancho Bernardo Study participants, women aged 40+ (n = 639)	824
	Depression	Hypo- and hypergonadal had higher depression scores than those with intermediate free testosterone	Androx Vienna Municipality Study participants, manual workers, men aged 43–67 (n = 689)	825
PTH	Mortality	~50% excess risk for individuals with low or high iPTH	Hemodialysis patients (n = 3946)	826
	Risk of vertebral or hip fractures	~33% higher for low or high iPTH compared to normal levels	Elderly dialysis patients (n = 9007)	827
TSH	Incidence of Alzheimer's disease	About double the incidence in lowest and highest tertile in women (no effects observed in men)	Framingham Study participants (elderly) (n = 1864, 59% women)	828
Leptin	Mortality	Mortality ~10% higher for lowest and highest leptin levels	Framingham Heart Study participants (elderly) (n = 818, 62% women)	563
Insulin	Coronary artery calcification	Higher for low and high insulin area under the curve measures.	Nondiabetic patients with suspected coronary heart disease, cross-sectional (n = 582)	829
	Mortality (noncardiovascular only)	Relative risk ~1.5 for highest and lowest fasting insulin levels	Helsinki Policemen Study participants, men aged 34–64 (n = 970)	830
Cortisol	BMI, waist circumference	Low cortisol secretion per hour for individuals with highest and lowest BMI, waist circumference	Whitehall II participants, adults, cross-sectional (n = 2915 men; n = 1041 women)	831
	Major depression (by diagnostic interview)	Slight increases at extremes of cortisol	Longitudinal Aging Study Amsterdam participants, aged 65+, cross-sectional (n = 1185)	832

BMI, Body mass index; iPTH, intact PTH; PTH, parathyroid hormone.

port the idea that NMDRCs are a fundamental feature of hormones. Importantly, it should be noted that most of the individuals surveyed in studies examining the effects of natural hormones have a disease status or are elderly. This of course does not mean that natural hormones induce NMDRCs in only these select populations but may instead be a reflection of the types of individuals available for these studies (for example, there are very few clinical events in younger people).

NMDRCs observed in the epidemiology literature from human populations exposed to EDCs are now starting to receive attention (Table 9). Here, most reports of NMDRCs come from studies of healthy individuals exposed to persistent organic pollutants POPs, chemicals that do not easily degrade and consequently bioaccumulate in human and animal tissues (542). These POPs do encompass a range of chemical classes including components of plastics, pesticides, and industrial pollutants. A large number of these studies have focused on endpoints that are relevant to metabolic disease, and together, these studies show that there is a recurring pattern of NMDRCs related to POPs and disease. Of course, not every study of POPs shows NMDRCs, and this is probably due to the distribution of EDCs in the populations examined.

In addition to the studies that show strong evidence for NMDRCs in human populations, there is also a subset of studies that provide suggestive evidence for nonmonotonic relationships between EDCs and human health endpoints (Table 9). In fact, the authors of many of these papers clearly identify U- or inverted U-shaped dose-response curves. However, when authors do not perform the appropriate statistical tests to verify the presence of a NMDRC, there is some ambiguity in their conclusions. The usual cross-sectional *vs.* prospective design dichotomy in epidemiology also is a factor that can influence the strength of a NMDRC, or prevent the detection of one at all. This disjunction in design is often incongruous with EDC exposure studies because we often know very little about clearance rates of the chemical, interactions with adiposity, and changes to these factors with age and gender. Yet regardless of any possible weaknesses in these studies, they provide supportive evidence that NMDRCs are observed in human populations.

Because these reports of NMDRCs in human populations are relatively new, few mechanisms have been proposed for these phenomena. Why would risk curves be nonmonotonic over the dose distribution observed in human populations? Why would individuals with the highest exposures have less severe health outcomes compared with individuals with more moderate exposures? One plausible explanation is that the same mechanisms for NMDRCs in animals and cell cultures operate in human

populations: chronic exposures to high doses can activate negative feedback loops, activate receptors that promote changes in different pathways that diverge on the same endpoint with opposing effects, or produce some measure of toxicity. Accidental exposures of very large doses may not behave the same as background doses for a variety of reasons, including the toxicity of high doses; these large doses tend to occur over a short time (and therefore more faithfully replicate what is observed in animal studies after controlled administration).

Another explanation is that epidemiology studies, unlike controlled animal studies, examine truly complex mixtures of EDCs and other environmental chemicals. Some chemical exposures are likely to be correlated due to their sources and their dynamics in air, water, soil, and living organisms that are subsequently eaten. Therefore, intake of these chemicals may produce unpredicted, likely nonlinear outcomes whether the two chemicals act via similar or different pathways.

The design of observational epidemiological studies is fundamentally different from studies of cells or animals, in that the EDC exposure distributions are given, rather than set by the investigator. In particular, as shown in Fig. 5, different epidemiological populations will have different ranges of exposure, with the schematic example showing increasing risk in a population with the lowest exposures (labeled group A), an inverted U-shaped risk in a moderate dose population (labeled group B), and an inverse risk in a population with the highest exposures (labeled group C). An additional example is provided (labeled group D) in which an industrial spill shows high risk, but the comparison with the entire unaffected population with a wide variety of risk levels due to differential background exposure could lead to a high- or a low-risk reference group and a wide variety of possible findings.

It is reasonable to suggest that even though epidemiological studies are an assessment of exposures at a single time point, many of these pollutants are persistent, and therefore a single measure of their concentration in blood may be a suitable surrogate for long-term exposures. The movement of people from relatively low- to higher-exposure groups over time depend on refreshed exposures, clearance rates, and individual differences in ability to handle exposures (*i.e.* due to genetic susceptibilities, amount of adipose tissue where POPs can be stored, *etc.*).

Figure 5 therefore further illustrates that observational epidemiological studies yield the composite effect of varying mixtures of EDCs at various exposure levels for various durations, combining acute and chronic effects. These studies are important, however, in that they are the only way to study EDC effects in the long term in intact humans, as opposed to studying signaling pathways, cells,

TABLE 9. NMDRCs for EDCs identified in the epidemiology literature

Chemicals by chemical class	Affected endpoint	NMDRC	Study subjects	Refs.
Insecticides				
Trans-nonachlor	Diabetes incidence	Highest risk in groups with intermediate exposures (quartile 2)	CARDIA participants, case-control study (n = 90 cases and n = 90 controls)	833
	Telomere length in peripheral leukocytes	Increased length in intermediate exposures (quintile 4)	Adults aged 40+ (Korea, n = 84)	591
p,p'-DDE	BMI, triglyceride levels, HDL cholesterol	Highest risk in groups with intermediate exposures (quartile 3)	CARDIA participants (n = 90 controls from nested case control study)	590
	Risk of rapid infant weight gain	For infants born to women of normal weight prepregnancy, risk is highest with intermediate exposures.	Infants from Childhood and the Environment project, Spain (n = 374 from normal prepregnancy weight mothers; n = 144 from overweight mothers)	834
	Telomere length in peripheral leukocytes	Increased length with intermediate exposures (quintile 4)	Adults aged 40+ (Korea, n = 84)	591
Oxychlorthane	Bone mineral density of arm bones	With low exposures, fat mass had inverse associations with bone mineral density; with high exposures, fat mass had positive associations with bone mineral density.	NHANES 1999–2004 participants, aged 50+ (n = 679 women, n = 612 men)	835
Plastics				
Mono-methyl phthalate (MMP)	Atherosclerotic plaques	Increased risk in intermediate exposure groups (quintiles 2–4)	Adults aged 70, living in Sweden (n = 1016)	836
Perfluorinated compounds				
PFOA	Arthritis (self-reported)	Increased risk in intermediate exposure groups (quartile 2)	NHANES participants, aged 20+ (both sexes, n = 1006)	837
Fire retardants				
PBB-153	Blood triglyceride levels	Increased risk in intermediate exposure groups (quartile 2)	NHANES participants, aged 12+ (n = 637)	604
PBDE-153	Prevalence of diabetes,	Prevalence of diabetes highest in intermediate groups (quartiles 2–3 relative to individuals with undetectable levels)	NHANES participants, aged 12+ (n = 1367)	604
	Prevalence of metabolic syndrome, levels of blood triglycerides	Prevalence of metabolic syndrome highest in intermediate exposure groups (quartile 2 relative to individuals with undetectable levels); blood triglycerides highest in low exposure groups (quartile 1 relative to individuals with undetectable levels)	NHANES participants, aged 12+ (n = 637)	604
PCB				
PCB-74	Triglyceride levels	Lowest levels are observed in intermediate groups (quartile 2)	CARDIA participants (n = 90 controls from nested case-control study)	590
PCB-126	Bone mineral density in right arm	With low exposures, fat mass had inverse associations with bone mineral density; with high exposures, fat mass had positive associations with bone mineral density	NHANES participants, aged <50 (n = 710 women, n = 768 men)	835
PCB-138	Bone mineral density in right arm	With low exposures, fat mass had inverse associations with bone mineral density; with high exposures, fat mass had positive associations with bone mineral density	NHANES participants, women aged 50+ (n = 679 women, n = 612 men)	835
PCB-153	Telomere length in peripheral leukocytes	Increased length with intermediate exposure groups (quintile 4)	Adults aged 40+ (Korea, n = 84)	591
PCB-170	Diabetes incidence	Highest risk in groups with intermediate exposures (quartile 2)	CARDIA participants, case-control study (n = 90 cases and n = 90 controls)	833
	Endometriosis	Decreased risk in groups with intermediate exposures (quartile 3)	Participants from the Women at Risk of Endometriosis (WREN) study, 18–49 yr old, case-control study (n = 251 cases; n = 538 controls)	838
PCB-172	DNA hypomethylation (by Alu assay)	Highest levels of hypomethylation in groups with lowest and highest exposures	Adults aged 40+ (Korea, n = 86)	839
PCB-180 ^a	BMI	Highest BMI with intermediate exposures (quartile 2)	CARDIA participants (n = 90 controls from nested case control study)	590
PCB-187 ^a	HDL cholesterol levels	Lowest levels with intermediate exposures (quartile 2)	CARDIA participants (n = 90 controls from nested case control study)	590
PCB 196–203	Diabetes incidence	Highest risk in groups with intermediate exposures (quartile 2)	CARDIA participants, case-control study (n = 90 cases and n = 90 controls)	833
PCB-196	Endometriosis	Decreased risk in groups with intermediate exposures (quartile 3)	Participants from the Women at Risk of Endometriosis (WREN) study, 18–49 yr old, case-control study (n = 251 cases; n = 538 controls)	838

(Continued)

TABLE 9. Continued

Chemicals by chemical class	Affected endpoint	NMDRC	Study subjects	Refs.
PCB-199 ^a	Triglyceride levels	Highest risk in groups with intermediate exposures (quartiles 2–3)	CARDIA participants (n = 90 controls from nested case control study)	590
PCB-201	Endometriosis	Decreased risk in groups with intermediate exposures (quartiles 2–3)	Participants from the Women at Risk of Endometriosis (WREN) study, 18–49 yr old, case-control study (n = 251 cases, n = 538 controls)	838
Heavy metals				
Selenium	Fasting glucose levels (by modeled exposure)	Intermediate exposures have highest fasting glucose levels	NHANES 2003–2004 participants, aged 40+ (n = 917)	840
	Glycosylated hemoglobin (by modeled exposure)	Intermediate exposures have highest % glycosylated hemoglobin	NHANES 2003–2004 participants, aged 40+ (n = 917)	840
	Diabetes incidence (by modeled exposure)	Intermediate exposures have highest risk for diabetes	NHANES 2003–2004 participants, aged 40+ (n = 917)	840
	Blood triglyceride levels	Intermediate exposures have highest triglyceride levels	NHANES participants, aged 40+ (n = 1159)	841
Arsenic	Cytokines in umbilical cord blood	Lower inflammatory markers at intermediate exposures (quartile 2)	Pregnant women in Bangladesh (n = 130)	842
Manganese	Mental development scores in infants and toddlers	Intermediate exposures had highest mental development scores at 12 months of age; association lost in older toddlers	12-month-old infants, Mexico (n = 301)	843
	Sperm count, motility and morphology	Intermediate doses had lowest sperm counts and motility; intermediate doses also had the worst sperm morphologies	Men aged 18–55 (infertility clinic patients, n = 200)	844
Mixtures				
31 POP	Diabetes incidence	Highest incidence in intermediate groups (sextiles 2–3)	CARDIA participants, case-control study (n = 90 cases and n = 90 controls)	833
16 POP	Diabetes incidence	Highest incidence in intermediate groups (sextiles 2–3)	CARDIA participants, case-control study (n = 90 cases and n = 90 controls)	833
Non-dioxin-like PCB (mix)	Metabolic syndrome	Highest incidence in intermediate groups (quartile 3)	NHANES 1999–2002 participants, aged 20+ (n = 721)	845
Dioxin-like PCB (mix)	Triacylglycerol levels by quartile of exposure	Highest levels in intermediate groups (quartile 3)	NHANES 1999–2002 participants, aged 20+ (n = 721)	845
Additional supportive evidence for NMDRC in the epidemiology literature				
Insecticides				
Heptachlor epoxide	Prevalence of newly diagnosed hypertension	Highest risk in intermediate groups (quartile 2); other endpoints do not have NMDRC	NHANES participants, women aged 40+, cross-sectional (n = 51 cases, n = 278 total)	826
β -Hexachloro-cyclohexane	Triacylglycerol levels by quartile of exposure	Highest risk in intermediate group (quartile 2)	NHANES participants, aged 20+ (n = 896 men, 175 with metabolic syndrome)	845
Plastics				
Mono- <i>N</i> -butyl phthalate (MBP)	BMI, age-specific effects	Effects seen only in elderly participants (age 60–80); risk is lowest in quartile 3	NHANES male participants (n = 365; age 60–80)	470
Mono-benzyl phthalate (MBzP)	BMI, age-specific effects	Effects seen only in young participants (age 6–11); risk is highest in quartiles 2–3	NHANES participants (both sexes, n = 329 males; n = 327 females)	470
Flame retardants				
PFOA	Thyroid disease (self-reported)	Lowest risk in intermediate groups (quartile 3)	NHANES 1999–2000, 2003–2006 participants, males aged 20+ (n = 3974)	837
Dioxin and related compounds				
TCDD	Age at natural menopause	Highest for intermediate exposure group (quintile 4)	Highly exposed women; Seveso Women's Health Study participants (n = 616)	468
HCDD	Bone mineral density in right arm by quintile of fat mass	With low exposures, fat mass had inverse associations with bone mineral density; with high exposures, fat mass had positive associations with bone mineral density	NHANES participants, women aged 50+ (n = 679 women, n = 612 men)	835
Heavy metals				
Selenium	Prevalence of peripheral artery disease	Disease prevalence decreased in intermediate doses, then increased gradually with higher doses	NHANES participants, aged 40+ (n = 2062)	469

BMI, Body mass index; HCDD, hexachloro-dibenzo-p-dioxin; HDL, high-density lipoprotein. PCB, polychlorinated biphenyls; PFOA, perfluorooctanoic acid; PBB, polybrominated biphenyl; PBDE, polybrominated diphenyl ethers; POP, persistent organic pollutants

^a In many cases, multiple chemicals in the same class had similar effects. A few chemicals were selected to illustrate the observed effect. This list is not comprehensive.

organs, or animal models over limited periods of time. Causal inference is not done directly from the epidemiological study results; instead, it is done via combining information from the epidemiological observations with

findings from the detailed studies of pathways and animals.

We have suggested that NMDRCs are a fundamental and general feature of hormone action in cells and animals.

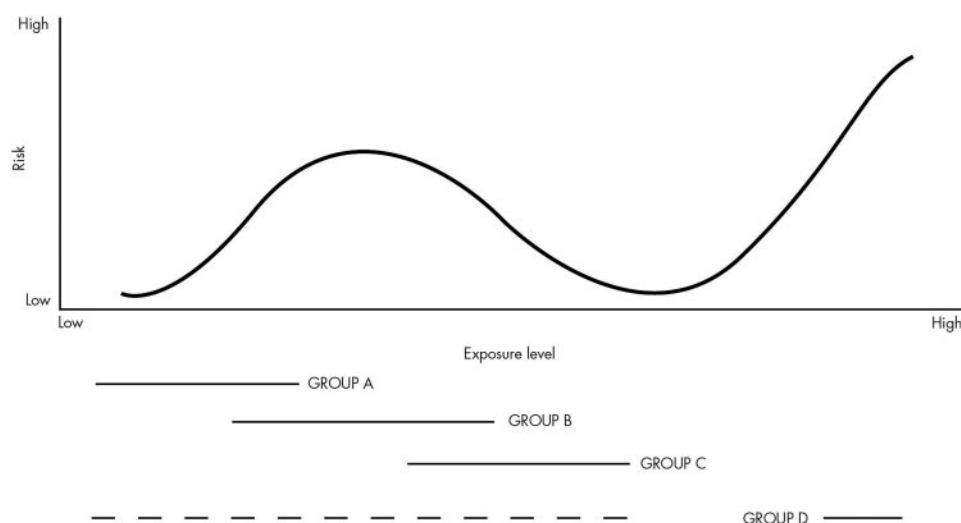
Figure 5.

Figure 5. Example of a NMDRC in humans and the sampling populations that could be examined in epidemiology studies. This schematic illustrates a theoretical NMDRC in a human population. If a study were to sample only group A, the conclusion would be that with increasing exposures, risk increases monotonically. Sampling group B would allow researchers to conclude that there is a nonmonotonic relationship between exposure level and risk. If a study included only group C, the conclusion would be that with increasing exposures, there is decreased risk of disease. Group D represents a population that was highly exposed, *i.e.* due to an industrial accident. This group has the highest risk, and there is a monotonic relationship between exposures and risk, although risk is high for all individuals. In the group D situation, there is generally a background population with which high-dose exposure is compared (*dotted line*); relative risk for group D would depend on whether that background population resembles group A, B, or C. From this example, it is clear that the population sampled could strongly influence the shape of the dose-response curve produced as well as the conclusions reached by the study.

It is therefore worth asking whether NMDRCs are expected in the epidemiology literature. The endpoints assessed in epidemiology studies are typically integrated effects, rather than short-term effects; therefore, the various cell- or organ-specific effects may cancel each other, particularly if they are NMDRCs (because they are unlikely to all have nonmonotonicity at the same dose and direction). Thus, NMDRCs are likely to be rarer in the epidemiology literature compared with studies examining the effects of a wide range of doses of an EDC on animals and cultured cells. Yet it is also important to ask what can be concluded if a NMDRC is detected in one epidemiology study but not in others examining the same chemical and outcome. There are several factors that must be considered. The first is that differences in the populations examined between the two studies could explain why a monotonic relationship is observed in one group and a nonmonotonic relationship in another (see Fig. 5). The second is that one or more studies may not be statistically designed to detect NMDRCs. Finally, it is plausible that the NMDRC is an artifact due to residual confounding or some other factor that was not considered in the experimental design. As more becomes known about the mechanisms operating in cells, tissues, and organs to generate NMDRCs, our ability to apply this information to epidemiology studies will increase as well.

4. Tamoxifen flare, a NMDRC observed in cells, animals, and human patients

Although there is controversy in toxicology and risk assessment for endocrine disruptors, NMDRCs are recognized and used in current human clinical practice, although under a different specific term, flare. Flare is often reported in the therapy of hormone-dependent cancers such as breast and prostate cancer. Clinically, failure to recognize the NMDRC that is termed a flare would be considered malpractice in human medicine.

Tamoxifen flare was described and named as a transient worsening of the symptoms of advanced breast cancer, particularly metastases to bone associated with increased pain, seen shortly after the initiation of therapy in some patients (543). If the therapy could be continued, the patients showing tamoxifen flare demonstrated a very high likelihood of subsequent response to tamoxifen, including arrest of tumor growth and progression of symptoms for some time.

The subsequent mechanism of the flare was described in basic lab studies in athymic mouse models of human hormone-dependent breast cancer xenografts (544) and in tissue culture of hormone-dependent human breast cancer cells (545–547). In these models, it was observed that although high, therapeutic concentrations of tamoxifen inhibited estrogen-stimulated proliferation of breast cancer cells, lower concentrations of tamoxifen actually stimulated breast can-

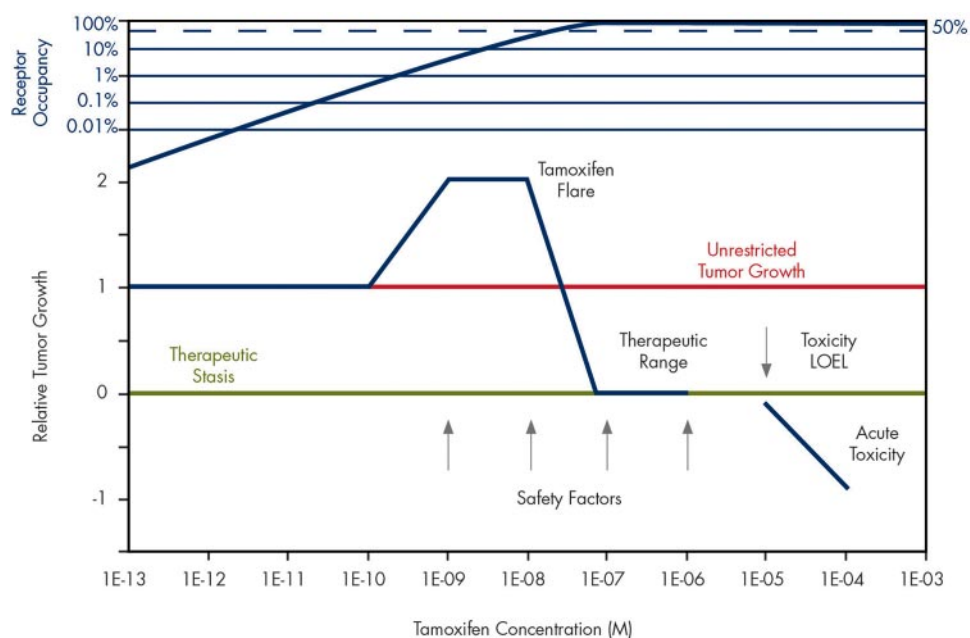
Figure 6.

Figure 6. Dose-response ranges for tamoxifen in breast cancer therapy. This figure demonstrates the NMDRC, also called flare, in tamoxifen treatments. As the circulating dose of tamoxifen increases when treatment starts, patients initially experience flare, *i.e.* growth of the tumor (546), followed by a decrease in tumor size as the circulating levels of tamoxifen rise into the therapeutic range (676, 677). High doses of tamoxifen are acutely toxic (546). Starting from the highest concentrations, where acute toxicity is observed, and going to lower concentrations on the X-axis, the acute toxicity diminishes towards zero growth, *i.e.* therapeutic stasis (*green* baseline). This occurs at approximately $1\text{E}-05$ m, the lowest observed effect level (LOEL) for toxicity. The vertical arrows show the results of applying three or four 10-fold safety factors to the LOEL for the high-dose toxicity of tamoxifen, and would calculate a safe or reference dose for tamoxifen in the region of flare, the least safe region of exposure in actual practice. Above the diagram of dose response ranges is estimated ER occupancy by tamoxifen. This was calculated from the affinity constant of tamoxifen for ERs determined in human breast cancer cells ($K_i = 29.1$ nM; Ref 678); flare appears to correspond to low receptor occupancy (*blue* axis), therapeutic range with mid and upper-range receptor occupancy, and acute toxicity well above 99% receptor occupancy. (678).

cer cell growth as long as the cells were estrogen dependent (548). Tamoxifen was also shown to disrupt tissue organization of the mammary gland, with specific effects on the stroma that may contribute to the observed effects on proliferation of epithelial cells (549, 550).

Tamoxifen therapy is administered as 10 mg twice per day (20 mg/d; approx 0.3 mg/kg body weight per day), but the target circulating levels are in the near submicromolar range (0.2–0.6 μM); these levels are reached slowly, after approximately 2 wks of therapy (551). In the initial period, where tamoxifen flare is observed, the circulating concentrations are ascending through lower concentrations, in the range below therapeutic suppression of growth, where breast cancer cell proliferation is actually stimulated by the drug, both in tissue culture, in animal xenograft studies, and in human patients (reviewed in Ref. 548). The recognition of this dual dose-response range for tamoxifen (low-dose, low-concentration estrogenic growth-stimulatory and higher-dose, higher-concentration estrogenic growth-inhibitory responses) led to the definition of the term selective estrogen response modu-

lator, or SERM, activity (552–554). This SERM activity has since been observed for many or even most estrogenic EDCs, including BPA (3, 555–557).

These observations defined three separate dose-response ranges for the SERM tamoxifen in human clinical use. The lowest dose-response range, the range of flare, stimulated breast cancer growth and symptoms in some patients with hormone-dependent cancer. The next higher dose-response range is the therapeutic range where tamoxifen inhibits estrogen-dependent tumor growth. The highest dose range causes acute toxicity by the SERM (see Fig. 6).

Tamoxifen provides an excellent example for how high-dose testing cannot be used to predict the effects of low doses. For tamoxifen (as for other drugs), the range of acute human toxicity for tamoxifen was determined in phase I clinical trials. Phase I trials also defined an initial therapeutic range, the second dose-response range, as a dose below which acute toxicity was not observed. The therapeutic dose range was tested and further defined in phase II and later clinical trials to determine efficacy (see for example Ref. 558). Standard toxicological testing from

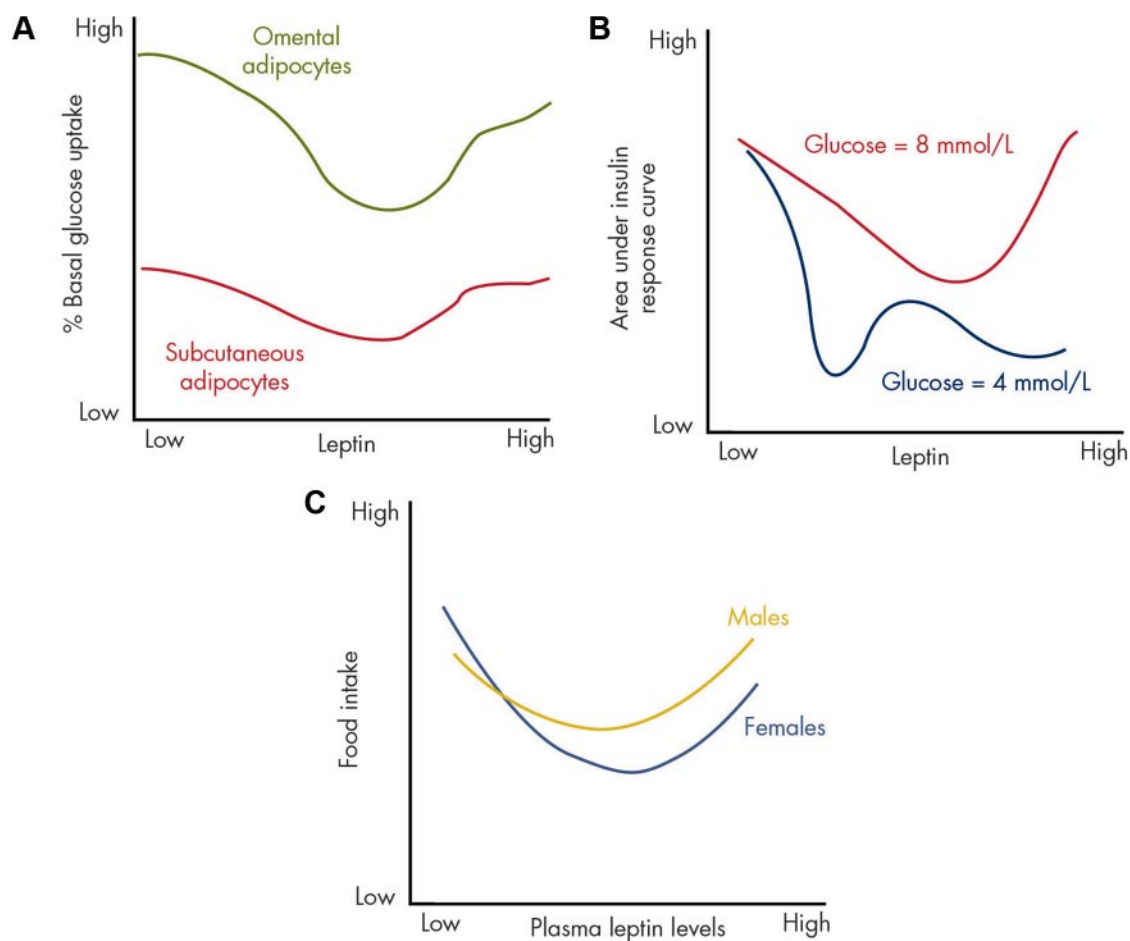
Figure 7.

Figure 7. Leptin as an example of a NMDRC. Several studies report NMDRCs in response to leptin treatments. A, NMDRCs are observed in cultured primary adipocytes after leptin exposure. This graph illustrates the relationship between administered leptin dose and glucose uptake in two types of adipocytes, those isolated from omental tissue (green) and others from sc fat (purple) (schematic was made from data in Ref. 559). These data are on a log-linear plot. B, *Ex vivo* rat pancreas was treated with leptin and various doses of glucose, and the insulin response curves were examined. Area under the curve is a measure of the ability of the pancreas to bring glucose levels under control. Different dose-response curves were observed depending on the amount of glucose administered: a U-shaped curve when 8 mmol/liter was included (pink) or a multiphasic curve with 4 mmol/liter (blue) (schematic made from data in Ref. 560). These data are on a linear-linear plot. C, U-shaped NMDRCs were also observed when food intake was compared with leptin levels in the blood of rats administered the hormone. This response was similar in males (orange) and females (cyan) (schematic made from data in Ref. 562). These data are on a linear-linear plot.

high doses to define a LOAEL or NOAEL are equivalent to the phase I clinical testing, and in risk assessment, a safe dose or reference dose is calculated from these tests. However, the lowest dose range, with the highly adverse effects termed flare, was not detected in the phase I trials and was determined only for tamoxifen in breast cancer therapy at the therapeutic doses (543). The implication for risk assessment is that NMDRCs for EDCs, particularly those already identified as SERMs, would likely not be detected by standard toxicological testing at high doses. That is, the consequence of high-dose testing is the calculation of a defined but otherwise untested safe dose that is well within the range equivalent to flare, *i.e.* a manifestly unsafe dose of the EDC (Fig. 6).

5. Similarities in endpoints across cell culture, animal, and epidemiology studies: evidence for common mechanisms?

There are common trends in some findings of NMDRCs in cell, animal, and human studies and therefore evidence for related mechanisms for NMDRCs at various levels of biological complexity. Tamoxifen flare, discussed in Section III.C.4, is an informative example. Another illustrative example is that of the effect of the hormone leptin (Fig. 7). In cultured primary adipocytes, NMDRCs are observed after leptin exposure; moderate doses of leptin significantly reduce insulin-mediated glucose intake, whereas low and high doses maintain higher glucose intake in response to insulin (559). The rat pancreas shows a similar response to leptin; the amount of

secreted insulin has an inverted U-shaped response to leptin (560, 561). Even more striking is the relationship between leptin and food intake. Rats administered moderate doses of leptin consume less food compared to rats dosed with low or high levels of leptin (562); mechanistically, this lower food intake could be due to higher circulating glucose levels in these animals due to ineffective insulin action. And finally, in a human study, leptin levels were found to correlate with body mass index but have a U-shaped relationship with mortality (563). These results suggest that hormones can produce similar responses at several levels of biological complexity (cell, organ, animal, and population).

A large number of epidemiology studies with NMDRCs have found relationships between EDC exposures like POPs and metabolic diseases including obesity and diabetes (Table 9) (see also Ref. 564 for a review), and the mechanisms for these relationships have begun to be explored. Human and animal cells treated with EDCs in culture display NMDRCs that are relevant to these diseases: BPA has nonmonotonic effects on the expression of adipocyte proteins in preadipocytes and the release of adiponectin from mature adipocytes (565–567). Similarly, in female rodents, low doses but not high doses of BPA increased adipose tissue weight and serum leptin concentrations (568), and intermediate doses of phthalates decrease serum cholesterol levels (569). Thus, although understanding the mechanisms operating at the cellular level of organization has not yet led to definitive knowledge of the mechanisms producing NMDRCs in human populations, there appear to be strong similarities in cells, animals, and humans that support a call for continued work focusing on metabolic disease endpoints at each level of biological organization.

D. NMDRC summary

We have demonstrated that nonmonotonicity is a common occurrence after exposures to hormones and EDCs in cell culture and animals and across human populations. Because of the abundance of examples of NMDRCs, we expect that if adequate dose ranges are included in animal and cell culture studies, including the use of negative and well-chosen positive controls, NMDRCs may be observed more often than not. Here, we have focused mainly on studies that examined a wide range of doses, including many that examined the effects of doses that span the low-dose and toxicological ranges. We also discussed several mechanisms that produce NMDRCs. Each of these mechanisms can and does operate at the same time in a biological system, and this cooperative action is ultimately responsible for NMDRCs.

Understanding nonmonotonicity has both theoretical and practical relevance. When a chemical produces mono-

tonic responses, all doses are expected to produce similar effects whose magnitude varies with the dose, but when a chemical produces a NMDRC, dissimilar or even opposite effects will be observed at different doses. Thus, monotonic responses can be modeled using the assumption that each step in a linear pathway behaves according to the law of mass action (43, 570); high doses are always expected to produce higher responses. In contrast, NMDRCs are not easy to model (although they are quite easy to test for), requiring detailed knowledge of the specific mechanisms operating in several biological components. From a regulatory standpoint, information from high doses cannot always be used to assess whether low doses will produce a biological effect (38).

IV. Implications of Low-Dose Effects and Nonmonotonicity

Both low-dose effects and NMDRCs have been observed for a wide variety of EDCs as well as natural hormones. Importantly, these phenomena encompass every level of biological organization, from gene expression, hormone production, and cell number to changes in tissue architecture to behavior and population-based disease risks. One conclusion from this review is that low-dose effects and NMDRCs are often observed after administration of environmentally relevant doses of EDCs. For both hormones and EDCs, NMDRCs should be the default assumption absent sufficient data to indicate otherwise. Furthermore, there are well-understood mechanisms to explain how low-dose effects and NMDRCs manifest *in vitro* and *in vivo*. Accepting these phenomena, therefore, should lead to paradigm shifts in toxicological studies and will likely also have lasting effects on regulatory science. Some of these aspects are discussed below. Additionally, we have briefly explored how this knowledge should influence future approaches in human and environmental health.

At a very practical level, we recommend that researchers publishing data with low-dose and nonmonotonic effects include key words in the abstract/article that identify them as such specifically. This review was unquestionably impeded because this has not been standard practice. We also strongly recommend that data showing nonmonotonic and binary response patterns not be rejected or criticized because there is no dose response.

A. Experimental design

1. Dose ranges must be chosen carefully

To detect low-dose effects or NMDRCs, the doses included for testing are of utmost importance. Most of the studies we examined here for nonmonotonicity tested

doses over severalfold concentrations. Unfortunately, regulatory guidelines only require that three doses be tested. Both low-dose effects and NMDRCs can be observed when examining only a few doses, but some studies may detect significant results purely by luck, because a small shift in dose can have a large impact on the ability to observe differences relative to untreated controls.

In the multitude of chemicals that have never been tested at low doses, or in the development of new chemicals, to determine whether a chemical has low-dose effects in laboratory animals, we suggest setting the NOAEL or LOAEL from traditional toxicological studies as the highest dose in experiments specifically designed to test endocrine-sensitive endpoints. We suggest setting the lowest dose in the experiment below the range of human exposures, if such a dose is known. Several intermediate doses overlapping the range of typical human exposures should be included also, bringing the total number in the range of five to eight total doses tested. Importantly, although the levels of many environmental chemicals in human blood and/or urine have been reported by the CDC and other groups responsible for population-scale biomonitoring, it is often not known what administered doses are needed to achieve these internal exposure levels in animals (4, 253); thus, toxicokinetic studies are often needed before the onset of low-dose testing. This is important because the critical issue is to determine what effects are observed in animals when circulating levels of an EDC match what is measured in the typical human. Due to differences in metabolism, route of exposure, and other factors, a relatively high dose may need to be administered to a rodent to produce blood concentrations in the range of human levels; however, this should not be considered a high-dose study.

It has also been suggested that animal studies that are used to understand the potential effects of a chemical on humans should use a relevant route of administration to recapitulate human exposures (571, 572) because there may be differences in metabolism after oral and nonoral administration. Many chemicals that enter the body orally undergo first-pass metabolism and are then inactivated via liver enzymes, whereas other routes (*i.e.* sc) can bypass these mechanisms and lead to a higher concentration of the active compound in circulation (573). Studies indicate, however, that inactivation of chemicals via first-pass metabolism is not complete and also that deconjugation of metabolites can occur in some tissues allowing the re-release of the active form (574, 575). Additionally, for some chemicals, it is clear that route of administration has little or no impact on the availability of the active compound in the body (241, 384), and other studies show that route of administration has no impact on the biological effects of

these chemicals; *i.e.* regardless of how it enters the body, dioxin has similar effects on exposed individuals (384), and comparable results have been observed for BPA (141). Although understanding the typical route of human exposure to each environmental chemical is an important task, it has been argued that any method that leads to blood concentrations of a test chemical in the range they are observed in humans is an acceptable exposure protocol, and this is especially true with gestational exposures, because fetuses are exposed to chemicals only via their mothers' blood (31, 576).

2. Timing of exposures is important

Rodent studies indicate that EDC exposures during development have organizational effects, with permanent effects that can manifest even in late adulthood, whereas exposures after puberty are for the most part activational, with effects that are abrogated when exposures cease. For example, the adult uterus requires relatively large doses of BPA (in the parts-per-million range) to induce changes associated with the uterotrophic assay (555, 577), whereas parts-per-trillion and ppb exposures during the fetal period permanently and effectively alter development of the uterus (279, 310, 578). Thus, the timing of exposures is profoundly important to detect low-dose effects of EDCs.

Human studies also support this conclusion. The 1976 explosion of a chemical plant in Seveso, Italy, which led to widespread human exposure to large amounts of TCDD, a particularly toxic form of dioxin, and the deposition of this chemical on the land surrounding the chemical plant, provided evidence in support of the organizational and activational effects of endocrine-active chemicals in humans (579). Serum TCDD concentrations showed correlations between exposure levels and several disease outcomes including breast cancer risk, abnormal menstrual cycles, and endometriosis (580–582), but individuals who were either infants or teenagers at the time of the explosion were found to be at greatest risk for developing adult diseases (583, 584). Importantly, many scientists have argued that organizational effects can occur during puberty, *i.e.* that the period where hormones have irreversible effects on organ development extends beyond the fetal and neonatal period (585), and for some endpoints this appears to be the case (586, 587).

It has also been proposed that the endocrine system maintains homeostasis in the face of environmental insults (210). The adult endocrine system does appear to provide some ability to maintain a type of homeostasis; when the pharmaceutical estrogen DES is administered to pregnant mice, the circulating estradiol concentrations in the dam respond by decreasing linearly (224). In contrast, fetal concentrations of estradiol respond nonmonotonically in

a way that is clearly not correlated with maternal levels. Similarly, there is evidence that BPA can induce aromatase and therefore increase estradiol levels *in situ* in the fetal urogenital sinus (588). This is an example of a feed-forward positive-feedback effect rather than a homeostatic response. The effects of EDCs on adult subjects, both animal and people, suggest that diseases often result from low-dose adult exposures (589–595); this argues against a view of the endocrine system as a means to maintain homeostatic control. Instead, individuals can be permanently changed, in an adverse way, after EDC exposures.

In one example, pregnant mice were exposed to low concentrations of BPA, and their male offspring had altered pancreatic function at 6 months of age (158). Surprisingly, however, the mothers (exposed only during pregnancy) were also affected, with altered metabolic machinery and body weight at 4 months postpartum, long after exposures had ended. The increased incidence of breast cancer in women that took DES during pregnancy also illustrates this point (596, 597). These studies suggest that even the adult endocrine system is not invariably capable of maintaining a so-called homeostatic state when exogenous chemicals affecting the endocrine system are present. Thus, although adult exposures to EDCs have been given some attention by bench scientists (29), more work of this kind is needed to better understand whether and how EDCs can have permanent organizational effects on adult animals.

At the beginning of this review, we justified the need to critically examine the low-dose literature because of recent epidemiological findings linking EDC exposures and diseases. Yet there is inherent difficulty in examining neonatal exposures to EDCs and their connection to diseases due to the length of time needed for these studies; thus, many studies of this type have examined high doses of pharmaceuticals (*i.e.* DES) or accidental exposures to industrial chemicals (*i.e.* dioxin) (66, 398, 399, 581, 597–601).

Only recently, with the availability of biomonitoring samples from large reference populations, have lower doses begun to receive widespread attention from epidemiologists. Many recent studies have examined adult exposures to EDCs and correlated exposures with disease statuses (see for example Refs. 15, 16, and 602–604). Human studies examining fetal/neonatal exposures to low-dose EDCs and early life effects have also begun to be studied (6, 333, 605–607), although studies linking these early life exposures to adult diseases are likely to be decades away. More than anything, these studies support our view that the effects of low-dose exposures should be considered when determining chemical safety.

3. Importance of endpoints being examined

Traditional toxicology testing, and in particular those studies performed for the purposes of risk assessment, typically adhere to guideline studies that have been approved by international committees of experts (608). The endpoints assessed in these guideline-compliant studies are centered around higher-order levels, including death, weight loss, mortality, and changes in organ weight, and a limited number of histopathological analyses (609, 610). When pregnant animals are included in toxicological assessments, the endpoints measured typically include the ability to maintain pregnancies, the number of offspring delivered, sex ratios of surviving pups, and measures regarding maternal weight gain and food/water intake (610).

Yet low-dose EDCs are rarely toxic to the point of killing adult animals or causing spontaneous abortions, and traditional tests such as the uterotrophic assay have been shown to be relatively insensitive (72, 577). It has been argued that this type of testing is insufficient for understanding the effects of EDCs (31, 70, 495, 611). Many EDC studies have instead focused on examining newly developed, highly sensitive endpoints that span multiple levels of biological organization, from gene expression to tissue organization to organ systems to the whole animal (612), which may not be rapidly lethal but which nonetheless have enormous importance for health, including mortality. Thus, for example, studies designed to examine the effects of chemicals on obesity no longer focus on body weight alone but also analyze gene expression; fat content in adipose cells and the process of adipogenesis; inflammation, innervation, and vascularization parameters in specific fat pads; conversion rates of white and brown adipose tissues; systemic hormone levels and response to glucose and insulin challenges; and food intake and energy expenditures, among others (314, 613–615). As our knowledge of EDCs and the endocrine system continue to grow, the most sensitive endpoints should be used to determine whether a chemical is disrupting the development of organisms (70).

In moving beyond traditional, well-characterized health-related endpoints like mortality and weight loss, an important question has been raised: how do we define endpoints as adverse? This is an important point, because it has been suggested that the creative endpoints examined in independent EDC studies are not validated and may not represent adverse effects (609). There is also debate over whether the mechanism (or mode) of action must be explained for each effect to determine whether a relevant pathway is present in humans (616, 617). Yet, when originally assessing the low-dose literature, the NTP expert panel chose to examine all effects of EDC exposure, re-

ardless of whether the endpoint could be deemed adverse (2). From the perspective of developmental biology, any change in development should be seen as adverse, even if the change itself is not associated with a disease or dysfunction. Some of these developmental changes, in fact, may increase sensitivity or susceptibility to disease later on in life but will otherwise appear normal. Furthermore, studies of heavy metals have shown that small shifts in parameters like IQ may not have drastic effects on individuals but can have serious repercussions on the population level (618), and therefore changes in the variance/observable range of a phenotype should also be considered adverse (52).

4. Importance of study size

National Institutes of Health guidelines require that the number of vertebrate animals used in experiments be as small as possible to show statistically significant effects based on power analysis. Yet many traditional toxicology studies have used large numbers of animals to draw conclusions about chemical safety. When the endpoints being assessed have binary outcomes (*i.e.* animal has a tumor *vs.* animal does not have a tumor) and the incidence of the phenotype is not high, a large number of animals is required to reveal statistically significant effects. In contrast, many of the endpoints examined in the field of endocrine disruption are more complex and are not binary; thus, power analysis allows researchers to determine how many animals are needed to observe statistically significant (and biologically relevant) differences between control and exposed populations. For this reason, arbitrary numbers set as cutoffs for determining whether a study is acceptable or unacceptable for risk assessments are not appropriate. Instead, the number of animals required for a study to be complete is dependent on the effect size, precision/variance, minimal meaningful difference to be considered between populations, and the α -value set in statistical tests.

B. Regulatory science

For decades, regulatory agencies have tested, or approved testing, of chemicals by examining high doses and then extrapolating down from the NOAEL, NOEL, and LOAEL to determine safe levels for humans and/or wildlife. As discussed earlier, these extrapolations use safety factors that acknowledge differences between humans and animals, exposures of vulnerable populations, interspecies variability, and other uncertainty factors. These safety factors are informed guesses, not quantitatively based calculations. Using this traditional way of setting safe doses, the levels declared safe are never in fact tested. Doses in the range of human exposures are therefore also unlikely to be tested. This has generated the current state of science,

where many chemicals of concern have never been examined at environmentally relevant low doses (see Table 4 for a small number of examples).

Assumptions used in chemical risk assessments to estimate a threshold dose below which daily exposure to a chemical is estimated to be safe are false for EDCs. First, experimental data provide evidence for the lack of a threshold for EDCs (619). More broadly, the data in this review demonstrate that the central assumption underlying the use of high doses to predict low-dose effects will lead to false estimates of safety. The use of only a few high doses is based on the assumption that all dose-response relationships are monotonic and therefore that it is appropriate to apply a log-linear extrapolation from high-dose testing to estimate a safe reference dose (Fig. 4). The Endocrine Society issued a position statement on EDCs (620) and urged the risk assessment community to use the expertise of their members to develop new approaches to chemical risk assessments for EDCs based on principles of endocrinology. Undertaking this mission will represent a true paradigm shift in regulatory toxicology (79). The Endocrine Society statement was then supported in March 2011 by a letter to *Science* from eight societies with relevant expertise representing over 40,000 scientists and medical professionals (621).

Studies conducted for the purposes of risk assessment are expected to include three doses: a dose that has no effects on traditional toxicological endpoints (the NOAEL), a higher dose with effects on traditional endpoints (the LOAEL), and an even higher dose that shows toxicity. Although reducing the number of animals used for these types of studies is an important goal, more than three doses are often needed for a true picture of a chemical's toxicity. The examination of a larger number of doses would allow for 1) the study of chemicals at the reference dose, *i.e.* the dose that is calculated to be safe; 2) examination of doses in the range of actual human exposures, which is likely to be below the reference dose; and 3) the ability to detect NMDRCs, particularly in the low-dose range. The impact of testing more doses on the numbers of animals required can be mitigated by use of power analysis, as suggested above. Because no amount of research will ever match the diversity and reality of actual human experience, there should be ongoing epidemiological study of potential adverse effects of EDCs even after safe levels are published, with periodic reevaluation of those safe levels.

One issue that has been raised by regulatory agencies is whether animal models are appropriate for understanding the effects of EDCs on humans. These arguments largely center around observed differences in hormone levels during different physiological periods in rodents and humans (57), and differences in the metabolic machinery and ex-

cretion of chemicals between species (622). To address the first issue, it should be noted that the FDA uses animals to test pharmaceuticals and other chemicals before any safety testing in humans because it is widely recognized that, although animals and humans do not have exactly the same physiologies, there is evolutionary conservation among vertebrates and specifically among mammals (62). Furthermore, animal studies proved to be highly predictive of the effects of DES on women, indicating that rodents are sufficiently similar to humans to reliably forecast affected endpoints in the endocrine system (64, 623). Thus, the default position must be that animal data are indicative of human effects until proven otherwise.

With regard to the second issue, BPA researchers in particular have examined species-specific differences in metabolism of this EDC. Interestingly, the pharmacokinetics of BPA in rodents, monkeys, and humans appear to be very similar (624), and regulatory agencies have subsequently concluded that rodents are appropriate models to assess the effects of this chemical (625, 626). Thus, researchers should select animal models that are sensitive to low doses of hormones and select appropriate species for the endpoints of interest. As the scope of our knowledge has broadened about how chemicals can alter the endocrine system, well beyond estrogens, androgens, and the thyroid, it is imperative that considerable thought be given to how to apply this for regulatory purposes.

C. Human health

As discussed several times throughout this review, there is now substantial evidence that low doses of EDCs have adverse effects on human health. Thus, although many epidemiological studies originally focused on occupationally exposed individuals and individuals affected by accidental exposures to high doses of environmental chemicals, these recent studies have suggested wide-ranging effects of EDCs on the general population.

Importantly, human exposures are examples of true mixtures; dozens if not hundreds of environmental chemicals are regularly detected in human tissues and fluids (91), yet very little is known about how these chemicals act in combination (627). Several studies indicate that EDCs can have additive or even synergistic effects (143, 323, 628–630), and thus these mixtures are likely to have unexpected and unpredictable effects on animals and humans. The study of mixtures is a growing and complex field that will require considerable attention in the years ahead as knowledge of EDCs in the laboratory setting are applied to human populations (631, 632).

How much will human health improve by testing chemicals at low, environmentally relevant doses and using the results to guide safety determinations? Current testing

paradigms are missing important, sensitive endpoints; because they are often unable to detect NMDRCs, they cannot make appropriate predictions about what effects are occurring at low doses. At this time, it is not possible to quantify the total costs of low-dose exposures to EDCs. However, current epidemiology studies linking low-dose EDC exposures to a myriad of health problems, diseases, and disorders suggest that the costs of current low-dose exposures are likely to be substantial.

The weight of the available evidence suggests that EDCs affect a wide range of human health endpoints that manifest at different stages of life, from neonatal and infant periods to the aging adult. As the American population ages, healthcare costs continue to rise, and there are societal costs as well, with decreased quality of life concerns, decreases in work productivity due to illness or the need for workers to care for affected family members, and the psychological stresses of dealing with some outcomes like infertility. Thus, it is logical to conclude that low-dose testing, followed by regulatory action to minimize or eliminate human exposures to EDCs, could significantly benefit human health. This proposal effectively calls for greatly expanded research to give human communities feedback about themselves. It emanates from a view that human society benefits greatly from the many chemical compounds it uses but that extensive epidemiological surveillance and other focused research designs are needed to assure that the balance of risk/benefit from those chemicals is acceptable.

How much would human health benefit by a reduction in the use of EDCs? For some chemicals, minor changes in consumer habits or industrial practices can have drastic effects on exposures (633–636). Other chemicals like DDT that have been regulated in the United States for decades continue to be detected in human and environmental samples; the persistent nature of many of these agents suggests they may impact human health for decades to come. Even less-persistent chemicals like BPA are likely to remain in our environment long after a ban is enacted because of the large amounts of plastic waste leaching BPA (and other estrogenic compounds) from landfills into water sources (637) and its presence on thermal receipt paper and from there into recycled paper (638–640). Yet, despite these challenges, reducing human exposure to EDCs should be a priority, and one way to address that priority is to decrease the production and use of these chemicals. The Endocrine Society has called for such a reduction and the use of the precautionary principle, *i.e.* action in the presence of concerning information but in the absence of certainty to eliminate or cut the use of questionable chemicals even when cause-effect relationships are not yet established (620).

D. Wildlife

Much of the recent focus on EDCs has been on the impact of these chemicals on human health. Yet the earliest studies of EDCs that focused on the impact of these chemicals on wildlife should not be forgotten. Rachel Carson's work on DDT and other pesticides provided some of the earliest warning signs that there were unintended consequences of chemical use. Carson's work was ahead of its time; she understood that exceedingly small doses of these chemicals produced adverse effects, that the timing of exposures was critical, and that chemical mixtures produced compounded effects (641). Now, decades after some of the most dangerous EDCs have been regulated, they continue to be measured in environmental samples as well as the bodies of wildlife animals.

Furthermore, it should be pointed out that humans, like wildlife, are not insulated from the environment, and effects in wildlife, including nonmammalian species, are indicative of and mirror effects in humans. For example, BPA has estrogen-like effects in fish (642–644), amphibians (645, 646), and reptiles (647, 648). A recent review showed that demasculinizing and feminizing effects of atrazine have been demonstrated in fish, amphibians, reptiles, birds, and mammals, *i.e.* every vertebrate class examined (326); and in fact, the first report to suggest that atrazine induced aromatase was conducted in reptiles (649). Similarly, perchlorate affects fish (650–653), amphibians (654–658), and birds (659–661) via mechanisms consistent with those described for humans, and some of the earliest reports on perchlorate's effects on thyroid function were conducted in amphibians (661, 662). Finally, ecological studies of dioxin and dioxin-like chemicals reveal effects on a range of exposed wildlife including birds (663, 664), fish (665, 666), and invertebrates (667). Although these studies have highlighted some of the species-specific effects of dioxin (389), and orders of magnitude differences in toxic equivalency factors between species (668), they also indicate the conservation of mechanisms for the effects of dioxin on a range of biological endpoints in wildlife, laboratory animals, and humans (384). In fact, in many cases, nonmammalian species are much more sensitive to EDC effects, and wildlife species serve as sentinels for environmental and public health (669–673). Thus, the effects of these chemicals on wildlife populations are likely to continue; for this reason, the low-dose effects of these chemicals are particularly worth understanding (674, 675).

V. Summary

In conclusion, we have provided hundreds of examples that clearly show that NMDRCs and low-dose effects are

common in studies of hormones and EDCs. We have examined each of these issues separately and provided mechanistic explanations and examples of both. These topics are related, but they must be examined individually to be understood. The concept of nonmonotonicity is an essential one for the field of environmental health science because when NMDRCs occur, the effects of low doses cannot be predicted by the effects observed at high doses. In addition, the finding that chemicals have adverse effects on animals and humans in the range of environmental exposures clearly indicates that low doses cannot be ignored.

In closing, we encourage scientists and journal editors to publish data demonstrating NMDRCs and low-dose effects, even if the exact mechanism of action has not yet been elucidated. This is important because the study of EDC is a growing specialty that crosses many scientific fields, and scientists that work on or regulate EDCs should appreciate and acknowledge the existence of NMDRCs and low-dose effects and have access to this important information. We further recommend greatly expanded and generalized safety testing and surveillance to detect potential adverse effects of this broad class of chemicals. Before new chemicals are developed, a wider range of doses, extending into the low-dose range, should be fully tested. And finally, we envision that the concepts and empirical results we have presented in this paper will lead to many more collaborations among research scientists in academic and government laboratories across the globe, that more and more sophisticated study designs will emerge, that what we have produced herein will facilitate those making regulatory decisions, that actions taken in light of this information will begin to abate the use of EDCs, and ultimately that health impacts in people and in wildlife will be averted.

Acknowledgments

We thank many colleagues in the fields of endocrine disruption and environmental health science for fruitful discussions on the topics covered in this manuscript. We also thank three anonymous reviewers whose comments and suggestions greatly improved this review.

Address requests for reprints to: Laura N. Vandenberg, Tufts University, Center for Regenerative and Developmental Biology, 200 Boston Avenue, Suite 4600, Medford, Massachusetts 02155. E-mail: laura.vandenberg@tufts.edu; or J. P. Myers, Environmental Health Sciences, 421 Park Street, Charlottesville, Virginia 22902. E-mail: jpmymers@ehsciences.org.

This work was supported by National Institutes of Health (NIH) Grants GM 087107 (to L.N.V.), ES 08314 (to A.M.S.), ES 010026 (to R.T.Z.), ES018764 (to F.S.v.S.), HL 53560 (to D.R.J.), UMC MO-VMFC0018 (to W.V.W.), a Susan G. Komen for Cure Grant FAS0703860 (to T.S.), grants from the Mitchell Kapor Foundation, the

Cornell-Douglas Foundation, and the Wallace Global Fund (to T.B.H.) and a grant from the Kendeda Foundation (to J.P.M.). This article may be the work product of an employee or group of employees of the National Institute of Environmental Health Sciences (NIEHS), NIH; however, the statements, opinions or conclusions contained therein do not necessarily represent the statements, opinions or conclusions of NIEHS, NIH, or the U.S. government.

We dedicate this manuscript to Professor Howard A. Bern. Dr. Bern was an exceptionally brilliant biologist and a generous and inspiring colleague. His work spanning a wide range of organisms addressed multiple aspects of organismal and evolutionary biology. He was one of the founders of the field of comparative endocrinology and a pioneer in the study of endocrine disruption, anticipating the deleterious effects of developmental exposure to estrogens one decade before the discovery of the effects of diethylstilbestrol in women fetally exposed to this chemical. His pioneering work included, among other subjects, neuroendocrinology, reproduction, and mammary cancer. He was also an excellent mentor to many researchers who, in turn, advanced these endeavors. He left an indelible mark on all of us that had the privilege of meeting him.

Disclosure Summary: Fred vom Saal worked as a consultant and provided expert testimony (<\$10K). The authors have nothing to disclose.

References

1. **National Toxicology Program** 2001 National Toxicology Program's report of the endocrine disruptors low dose peer review. Research Triangle Park, NC: National Institute of Environmental Health Sciences
2. **Melnick R, Lucier G, Wolfe M, Hall R, Stancel G, Prins G, Gallo M, Reuhl K, Ho SM, Brown T, Moore J, Leakey J, Haseman J, Kohn M** 2002 Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. *Environ Health Perspect* 110:427–431
3. **Welshons WV, Nagel SC, vom Saal FS** 2006 Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology* 147:S56–S69
4. **Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV** 2007 Human exposure to bisphenol A (BPA). *Reprod Toxicol* 24:139–177
5. **Brucker-Davis F, Thayer K, Colborn T** 2001 Significant effects of mild endogenous hormonal changes in humans: considerations for low-dose testing. *Environ Health Perspect* 109:21–26
6. **Braun JM, Yolton K, Dietrich KN, Hornung R, Ye X, Calafat AM, Lanphear BP** 2009 Prenatal bisphenol A exposure and early childhood behavior. *Environ Health Perspect* 117:1945–1952
7. **Meeker JD, Barr DB, Hauser R** 2009 Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. *Reprod Toxicol* 27:155–160
8. **Weuve J, Hauser R, Calafat AM, Missmer SA, Wise LA** 2010 Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999–2004. *Environ Health Perspect* 118:825–832
9. **Meeker JD, Sathyanarayana S, Swan SH** 2009 Phthalates and other additives in plastics: human exposure and associated health outcomes. *Philos Trans R Soc Lond B Biol Sci* 364:2097–2113
10. **Swan SH** 2008 Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. *Environ Res* 108:177–184
11. **Akinbami LJ, Lynch CD, Parker JD, Woodruff TJ** 2010 The association between childhood asthma prevalence and monitored air pollutants in metropolitan areas, United States, 2001–2004. *Environ Res* 110:294–301
12. **Stillerman KP, Mattison DR, Giudice LC, Woodruff TJ** 2008 Environmental exposures and adverse pregnancy outcomes: a review of the science. *Reprod Sci* 15:631–650
13. **Grün F** 2010 Obesogens. *Curr Opin Endocrinol Diabetes Obes* 17:453–459
14. **Soto AM, Sonnenschein C** 2010 Environmental causes of cancer: endocrine disruptors as carcinogens. *Nat Rev Endocrinol* 6:363–370
15. **Meeker JD** 2010 Exposure to environmental endocrine disrupting compounds and men's health. *Maturitas* 66:236–241
16. **Hatch EE, Nelson JW, Stahlhut RW, Webster TF** 2010 Association of endocrine disruptors and obesity: perspectives from epidemiological studies. *Int J Androl* 33:324–332
17. **Hsu ST, Ma CI, Hsu SK, Wu SS, Hsu NH, Yeh CC, Wu SB** 1985 Discovery and epidemiology of PCB poisoning in Taiwan: a four-year followup. *Environ Health Perspect* 59:5–10
18. **Pesatori AC, Consonni D, Bachetti S, Zocchetti C, Bonzini M, Baccarelli A, Bertazzi PA** 2003 Short- and long-term morbidity and mortality in the population exposed to dioxin after the "Seveso accident". *Ind Health* 41:127–138
19. **Anderson HA, Wolff MS, Lilis R, Holstein EC, Valciukas JA, Anderson KE, Petrocci M, Sarkozi L, Selikoff IJ** 1979 Symptoms and clinical abnormalities following ingestion of polybrominated-biphenyl-contaminated food products. *Ann NY Acad Sci* 320:684–702
20. **Villeneuve S, Cyr D, Lyng E, Orsi L, Sabroe S, Merletti F, Gorini G, Morales-Suarez-Varela M, Ahrens W, Baumgardt-Elms C, Kaerlev L, Eriksson M, Hardell L, Févotte J, Guénel P** 2010 Occupation and occupational exposure to endocrine disrupting chemicals in male breast cancer: a case-control study in Europe. *Occup Environ Med* 67:837–844
21. **Li D, Zhou Z, Qing D, He Y, Wu T, Miao M, Wang J, Weng X, Ferber JR, Herrinton LJ, Zhu Q, Gao E, Checkoway H, Yuan W** 2010 Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction. *Hum Reprod* 25:519–527
22. **Queiroz EK, Waissmann W** 2006 Occupational exposure and effects on the male reproductive system. *Cad Saude Publica* 22:485–493
23. **Centers for Disease Control** 2008 National Biomonitoring Program. Atlanta, GA: Centers for Disease Control, Prevention
24. **Kuklennyik Z, Ye X, Needham LL, Calafat AM** 2009 Automated solid-phase extraction approaches for large scale biomonitoring studies. *J Chromatogr Sci* 47:12–18
25. **Umweltbundesamt** 2009 Health and environmental hygiene: German environmental survey. Umweltbundesamt Dessau-Rosslau, Berlin, Germany
26. **Ha MH, Lee DH, Son HK, Park SK, Jacobs Jr DR** 2009 Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hyper-

- tension: results from the National Health and Nutrition Examination Survey 1999–2002. *J Hum Hypertens* 23: 274–286
27. vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, Farabollini F, Guillette Jr LJ, Hauser R, Heindel JJ, Ho SM, Hunt PA, Iguchi T, Jobling S, Kanno J, Keri RA, Knudsen KE, Laufer H, LeBlanc GA, Marcus M, McLachlan JA, Myers JP, Nadal A, Newbold RR, Olea N, *et al.* 2007 Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. *Reprod Toxicol* 24:131–138
 28. Crain DA, Eriksen M, Iguchi T, Jobling S, Laufer H, LeBlanc GA, Guillette Jr LJ 2007 An ecological assessment of bisphenol-A: evidence from comparative biology. *Reprod Toxicol* 24:225–239
 29. Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenberg JG, Walser-Kuntz DR, vom Saal FS 2007 In vivo effects of bisphenol A in laboratory rodent studies. *Reprod Toxicol* 24:199–224
 30. Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM 2007 In vitro molecular mechanisms of bisphenol A action. *Reprod Toxicol* 24:178–198
 31. Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM 2009 Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. *Endocrine Reviews* 30:75–95
 32. Keri RA, Ho SM, Hunt PA, Knudsen KE, Soto AM, Prins GS 2007 An evaluation of evidence for the carcinogenic activity of bisphenol A. *Reprod Toxicol* 24:240–252
 33. U.S. Food and Drug Administration 2008 Draft assessment of bisphenol A for use in food contact applications. Washington, DC: Department of Health and Human Services
 34. U.S. Food and Drug Administration 2010 Update on bisphenol A (BPA) for use in food: January 2010. Washington, DC: Department of Health and Human Services
 35. Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO 1995 The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ Health Perspect* 103(Suppl 7):113–122
 36. Nagel SC, vom Saal FS, Welshons WV 1999 Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum. *J Steroid Biochem Mol Biol* 69:343–357
 37. Soto AM, Chung KL, Sonnenschein C 1994 The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. *Environ Health Perspect* 102:380–383
 38. Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS 2003 Large effects from small exposures: I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ Health Perspect* 111:994–1006
 39. Kochukov MY, Jeng YJ, Watson CS 2009 Alkylphenol xenoestrogens with varying carbon chain lengths differentially and potently activate signaling and functional responses in GH3/B6/F10 somatomammotropes. *Environ Health Perspect* 117:723–730
 40. Aleya RA, Watson CS 2009 Differential regulation of dopamine transporter function and location by low concentrations of environmental estrogens and 17 β -estradiol. *Environ Health Perspect* 117:778–783
 41. Wozniak AL, Bulayeva NN, Watson CS 2005 Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor- α mediated Ca²⁺ fluxes and prolactin release in GH3/B6 pituitary tumor cells. *Environ Health Perspect* 113:431–439
 42. Kohn MC, Melnick RL 2002 Biochemical origins of the non-monotonic receptor-mediated dose-response. *J Mol Endocrinol* 29:113–123
 43. Conolly RB, Lutz WK 2004 Nonmonotonic dose-response relationships: mechanistic basis, kinetic modeling, and implications for risk assessment. *Toxicol Sci* 77:151–157
 44. Zsarnovszky A, Le HH, Wang HS, Belcher SM 2005 Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. *Endocrinology* 146:5388–5396
 45. Wong JK, Le HH, Zsarnovszky A, Belcher SM 2003 Estrogens and ICI182,780 (Faslodex) modulate mitosis and cell death in immature cerebellar neurons via rapid activation of p44/p42 mitogen-activated protein kinase. *J Neurosci* 23:4984–4995
 46. Querfeld U, Mak RH 2010 Vitamin D deficiency and toxicity in chronic kidney disease: in search of the therapeutic window. *Pediatr Nephrol* 25:2413–2430
 47. Cook R, Calabrese EJ 2006 The importance of hormesis to public health. *Environ Health Perspect* 114:1631–1635
 48. Thayer KA, Melnick R, Huff J, Burns K, Davis D 2006 Hormesis: a new religion? *Environ Health Perspect* 114: A632–A633
 49. Weltje L, vom Saal FS, Oehlmann J 2005 Reproductive stimulation by low doses of xenoestrogens contrasts with the view of hormesis as an adaptive response. *Hum Exp Toxicol* 24:431–437
 50. Thayer KA, Melnick R, Burns K, Davis D, Huff J 2005 Fundamental flaws of hormesis for public health decisions. *Environ Health Perspect* 113:1271–1276
 51. Beronius A, Rudén C, Håkansson H, Hanberg A 2010 Risk to all or none? A comparative analysis of controversies in the health risk assessment of bisphenol A. *Reprod Toxicol* 29:132–146
 52. Bellinger DC 2004 What is an adverse effect? A possible resolution of clinical and epidemiological perspectives on neurobehavioral toxicity. *Environ Res* 95:394–405
 53. Foster PM, McIntyre BS 2002 Endocrine active agents: implications of adverse and non-adverse changes. *Toxicol Pathol* 30:59–65
 54. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL 2005 Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect* 113:1056–1061
 55. McEwen Jr GN, Renner G 2006 Validity of anogenital distance as a marker of *in utero* phthalate exposure. *Environ Health Perspect* 114:A19–A20; author reply A20–A21
 56. Weiss B 2006 Anogenital distance: defining “normal.” *Environ Health Perspect* 114:A399; author reply A399

57. Witorsch RJ 2002 Low-dose *in utero* effects of xenoestrogens in mice and their relevance to humans: an analytical review of the literature. *Food Chem Toxicol* 40:905–912
58. O'Lone R, Frith MC, Karlsson EK, Hansen U 2004 Genomic targets of nuclear estrogen receptors. *Mol Endocrinol* 18:1859–1875
59. Schulkin J 2011 Evolutionary conservation of glucocorticoids and corticotropin releasing hormone: behavioral and physiological adaptations. *Brain Res* 1392:27–46
60. Williams GR, Franklyn JA 1994 Physiology of the steroid-thyroid hormone nuclear receptor superfamily. *Baillieres Clin Endocrinol Metab* 8:241–266
61. Enmark E, Gustafsson JA 1999 Oestrogen receptors: an overview. *J Intern Med* 246:133–138
62. U.S. Food and Drug Administration 2009 Information for consumers (drugs). In: *The beginnings: laboratory and animal studies*. Washington, DC: Department of Health and Human Services
63. Mittendorf R 1995 Teratogen update: carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) *in utero*. *Teratology* 51:435–445
64. McLachlan JA 2006 Commentary: prenatal exposure to diethylstilbestrol (DES): a continuing story. *Int J Epidemiol* 35:868–870
65. Newbold RR, Jefferson WN, Padilla-Banks E 2007 Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. *Reprod Toxicol* 24:253–258
66. Palmer JR, Wise LA, Hatch EE, Troisi R, Titus-Ernstoff L, Strohshitter W, Kaufman R, Herbst AL, Noller KL, Hyer M, Hoover RN 2006 Prenatal diethylstilbestrol exposure and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 15:1509–1514
67. Soto AM, Vandenberg LN, Maffini MV, Sonnenschein C 2008 Does breast cancer start in the womb? *Basic Clin Pharmacol Toxicol* 102:125–133
68. Kamrin MA 2007 The “low dose” hypothesis: validity and implications for human risk. *Int J Toxicol* 26:13–23
69. Myers JP, vom Saal FS, Akingbemi BT, Arizono K, Belcher S, Colborn T, Chahoud I, Crain DA, Farabollini F, Guillette Jr LJ, Hassold T, Ho SM, Hunt PA, Iguchi T, Jobling S, Kanno J, Laufer H, Marcus M, McLachlan JA, Nadal A, Oehlmann J, Olea N, Palanza P, Parmigiani S, Rubin BS, et al. 2009 Why public health agencies cannot depend upon ‘Good Laboratory Practices’ as a criterion for selecting data: the case of bisphenol-A. *Environ Health Perspect* 117:309–315
70. Myers JP, Zoeller RT, vom Saal FS 2009 A clash of old and new scientific concepts in toxicity, with important implications for public health. *Environ Health Perspect* 117:1652–1655
71. vom Saal FS, Akingbemi BT, Belcher SM, Crain DA, Crews D, Giudice LC, Hunt PA, Lerner C, Myers JP, Nadal A, Olea N, Padmanabhan V, Rosenfeld CS, Schneyer A, Schoenfelder G, Sonnenschein C, Soto AM, Stahlhut RW, Swan SH, Vandenberg LN, Wang HS, Watson CS, Welshons WV, Zoeller RT 2010 Flawed experimental design reveals the need for guidelines requiring appropriate positive controls in endocrine disruption research. *Toxicol Sci* 115:612–613; author reply 614–620
72. vom Saal FS, Myers JP 2010 Good laboratory practices are not synonymous with good scientific practices, accurate reporting, or valid data. *Environ Health Perspect* 118:A60
73. Travis GD 1981 Replicating replication? Aspects of the social construction of learning in planarian worms. *Social Studies Sci* 11:11–32
74. Phillips CV, Goodman KJ 2004 The missed lessons of Sir Austin Bradford Hill. *Epidemiol Pespect Innov* 1:3
75. vom Saal FS, Hughes C 2005 An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. *Environ Health Perspect* 113:926–933
76. Hayes TB 2004 There is no denying this: defusing the confusion about atrazine. *BioScience* 54:1138–1149
77. vom Saal FS, Welshons WV 2006 Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A. *Environmental Research* 100:50–76
78. Bern HA, Edery M, Mills KT, Kohrman AF, Mori T, Larson L 1987 Long-term alterations in histology and steroid receptor levels of the genital tract and mammary gland following neonatal exposure of female BALB/cCrgl mice to various doses of diethylstilbestrol. *Cancer Res* 47:4165–4172
79. Krimsky S 2003 *Hormonal chaos: the scientific and social origins of the environmental endocrine hypothesis*. Baltimore: Johns Hopkins University Press
80. Barker DJ 2007 The origins of the developmental origins theory. *J Intern Med* 261:412–417
81. Barker DJP 2004 The developmental origins of adult disease. *J Am Coll Nutr* 23:588S–595S
82. Sharpe RM, Skakkebaek NE 1993 Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 341:1392–1395
83. Trichopoulos D 1990 Is breast cancer initiated *in utero*? *Epidemiology* 1:95–96
84. Heindel JJ 2006 Role of exposure to environmental chemicals in the developmental basis of reproductive disease and dysfunction. *Semin Reprod Med* 24:168–177
85. Crain DA, Janssen SJ, Edwards TM, Heindel J, Ho SM, Hunt P, Iguchi T, Juul A, McLachlan JA, Schwartz J, Skakkebaek N, Soto AM, Swan S, Walker C, Woodruff TK, Woodruff TJ, Giudice LC, Guillette Jr LJ 2008 Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing. *Fertil Steril* 90:911–940
86. Heindel JJ 2005 The fetal basis of adult disease: Role of environmental exposures: introduction. *Birth Defects Res A Clin Mol Teratol* 73:131–132
87. Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgarten FJ, Schoenfelder G 2010 Urine, serum and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environ Health Perspect* 118:1055–1070
88. Hays SM, Aylward LL 2009 Using biomonitoring equivalents to interpret human biomonitoring data in a public health risk context. *J Appl Toxicol* 29:275–288
89. Clewell HJ, Tan YM, Campbell JL, Andersen ME 2008 Quantitative interpretation of human biomonitoring data. *Toxicol Appl Pharmacol* 231:122–133
90. Hayes TB, Case P, Chui S, Chung D, Haeffele C, Haston K, Lee M, Mai VP, Marjuoa Y, Parker J, Tsui M 2006 Pesticide mixtures, endocrine disruption, and amphibian de-

- clines: are we underestimating the impact? *Environ Health Perspect* 114:40–50
91. Woodruff TJ, Zota AR, Schwartz JM 2011 Environmental chemicals in pregnant women in the US: NHANES 2003–2004. *Environ Health Perspect* 119:878–885
 92. Young SS, Yu M 2009 Association of bisphenol A with diabetes and other abnormalities. *JAMA* 301:720–721
 93. Smith GD, Ebrahim S 2002 Data dredging, bias, or confounding. *BMJ* 325:1437–1438
 94. Marshall JR 1990 Data dredging and noteworthiness. *Epidemiology* 1:5–7
 95. Vandembroucke JP 2008 Observational research, randomised trials, and two views of medical science. *PLoS Medicine* 5:e67
 96. Greenland S 2007 Commentary: on 'quality in epidemiological research: should we be submitting papers before we have the results and submitting more hypothesis generating research?'. *Int J Epidemiol* 36:944–945
 97. Melzer D, Lang IA, Galloway TS 2009 Reply to Young and Yu: association of bisphenol A with diabetes and other abnormalities. *JAMA* 301:721–722
 98. Wigle DT, Arbuckle TE, Turner MC, Bérubé A, Yang Q, Liu S, Krewski D 2008 Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. *J Toxicol Environ Health B Crit Rev* 11:373–517
 99. Watson CS, Gametchu B 1999 Membrane-initiated steroid actions and the proteins that mediate them. *Proc Soc Exp Biol Med* 220:9–19
 100. Frühbeck G 2006 Intracellular signalling pathways activated by leptin. *Biochem J* 393:7–20
 101. George JW, Dille EA, Heckert LL 2011 Current concepts of follicle-stimulating hormone receptor gene regulation. *Biol Reprod* 84:7–17
 102. Cheng SY, Leonard JL, Davis PJ 2010 Molecular aspects of thyroid hormone actions. *Endocr Rev* 31:139–170
 103. Kress E, Samarut J, Plateroti M 2009 Thyroid hormones and the control of cell proliferation or cell differentiation: paradox or duality? *Mol Cell Endocrinol* 313:36–49
 104. Fu M, Wang C, Zhang X, Pestell RG 2004 Acetylation of nuclear receptors in cellular growth and apoptosis. *Biochem Pharmacol* 68:1199–1208
 105. Katzenellenbogen BS, Montano MM, Ediger TR, Sun J, Ekena K, Lazennec G, Martini PG, McInerney EM, Delage-Mourroux R, Weis K, Katzenellenbogen JA 2000 Estrogen receptors: selective ligands, partners, and distinctive pharmacology. *Recent Prog Horm Res* 55:163–193; discussion 194–195
 106. Zhao C, Dahlman-Wright K, Gustafsson JA 2008 Estrogen receptor β : an overview and update. *Nucl Recept Signal* 6:e003
 107. Neill JD 2005 *Knobil and Neill's physiology of reproduction*. 3rd ed. New York: Academic Press
 108. Jones KA 1996 Summation of basic endocrine data. In: Gass GH, Kaplan HM, eds. *Handbook of endocrinology*. 2nd ed. New York: CRC Press; 1–42
 109. Stokes WS 2004 Selecting appropriate animal models and experimental designs for endocrine disruptor research and testing studies. *ILAR J* 45:387–393
 110. May M, Moran JF, Kimelberg H, Triggle DJ 1967 Studies on the noradrenaline α -receptor. II. Analysis of the "spare receptor" hypothesis and estimation of the concentration of α -receptors in rabbit aorta. *Mol Pharmacol* 3:28–36
 111. Zhu BT 1996 Rational design of receptor partial agonists and possible mechanisms of receptor partial activation: a theory. *J Theor Biol* 181:273–291
 112. Gan EH, Quinton R 2010 Physiological significance of the rhythmic secretion of hypothalamic and pituitary hormones. *Prog Brain Res* 181:111–126
 113. Naftolin F, Garcia-Segura LM, Horvath TL, Zsarnovszky A, Demir N, Fadiel A, Leranth C, Vondracek-Klepper S, Lewis C, Chang A, Parducz A 2007 Estrogen-induced hypothalamic synaptic plasticity and pituitary sensitization in the control of the estrogen-induced gonadotrophin surge. *Reprod Sci* 14:101–116
 114. Son GH, Chung S, Kim K 2011 The adrenal peripheral clock: glucocorticoid and the circadian timing system. *Front Neuroendocrinol* 32:451–465
 115. Urbanski HF 2011 Role of circadian neuroendocrine rhythms in the control of behavior and physiology. *Neuroendocrinology* 93:211–222
 116. National Research Council 1999 *Hormonally active agents in the environment*. Washington, DC: National Academy Press
 117. Eick GN, Thornton JW 2011 Evolution of steroid receptors from an estrogen-sensitive ancestral receptor. *Mol Cell Endocrinol* 334:31–38
 118. Sheehan DM 2000 Activity of environmentally relevant low doses of endocrine disruptors and the bisphenol A controversy: initial results confirmed. *Proc Soc Exp Biol Med* 224:57–60
 119. Hayes TB, Anderson LL, Beasley VR, de Solla SR, Iguchi T, Ingraham H, Kestemont P, Kniewald J, Kniewald Z, Langlois VS, Luque EH, McCoy KA, Muñoz-de-Toro M, Oka T, Oliveira CA, Orton F, Ruby S, Suzawa M, Tavera-Mendoza LE, Trudeau VL, Victor-Costa AB, Willingham E 2011 Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. *J Steroid Biochem Mol Biol* 127:64–73
 120. Beato M, Klug J 2000 Steroid hormone receptors: an update. *Hum Reprod Update* 6:225–236
 121. Watson CS, Bulayeva NN, Wozniak AL, Finnerty CC 2005 Signaling from the membrane via membrane estrogen receptor- α : estrogens, xenoestrogens, and phytoestrogens. *Steroids* 70:364–371
 122. Powell CE, Soto AM, Sonnenschein C 2001 Identification and characterization of membrane estrogen receptor from MCF7 estrogen-target cells. *J Steroid Biochem Mol Biol* 77:97–108
 123. Levin ER 2011 Extranuclear steroid receptors: roles in modulation of cell functions. *Mol Endocrinol* 25:377–384
 124. Levin ER 2009 Plasma membrane estrogen receptors. *Trends Endocrinol Metab* 20:477–482
 125. Thomas P, Dong J 2006 Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. *J Steroid Biochem Mol Biol* 102:175–179
 126. Kenealy BP, Keen KL, Terasawa E 2011 Rapid action of estradiol in primate GnRH neurons: The role of estrogen receptor α and estrogen receptor β . *Steroids* 76:861–866
 127. Watson CS, Bulayeva NN, Wozniak AL, Alyea RA 2007

- Xenoestrogens are potent activators of nongenomic estrogenic responses. *Steroids* 72:124–134
128. Ropero AB, Alonso-Magdalena P, Ripoll C, Fuentes E, Nadal A 2006 Rapid endocrine disruption: environmental estrogen actions triggered outside the nucleus. *J Steroid Biochem Mol Biol* 102:163–169
 129. Nadal A, Alonso-Magdalena P, Ripoll C, Fuentes E 2005 Disentangling the molecular mechanisms of action of endogenous and environmental estrogens. *Pflugers Arch* 449: 335–343
 130. Thomas P, Pang Y, Filardo EJ, Dong J 2005 Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* 146:624–632
 131. Nadal A, Ropero AB, Laribi O, Maillet M, Fuentes E, Soria B 2000 Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor α and estrogen receptor β . *Proc Natl Acad Sci USA* 97:11603–11608
 132. Tanabe N, Kimoto T, Kawato S 2006 Rapid Ca^{2+} signaling induced by bisphenol A in cultured rat hippocampal neurons. *Neuro Endocrinol Lett* 27:97–104
 133. Ruehlmann DO, Steinert JR, Valverde MA, Jacob R, Mann GE 1998 Environmental estrogenic pollutants induce acute vascular relaxation by inhibiting L-type Ca^{2+} channels in smooth muscle cells. *FASEB J* 12:613–619
 134. Walsh DE, Dockery P, Doolan CM 2005 Estrogen receptor independent rapid non-genomic effects of environmental estrogens on $[\text{Ca}^{2+}]$ in human breast cancer cells. *Mol Cell Endocrinol* 230:23–30
 135. Shioda T, Chesnes J, Coser KR, Zou L, Hur J, Dean KL, Sonnenschein C, Soto AM, Isselbacher KJ 2006 Importance of dosage standardization for interpreting transcriptomic signature profiles: evidence from studies of xenoestrogens. *Proc Natl Acad Sci USA* 103:12033–12038
 136. Ryan BC, Vandenberg JG 2002 Intrauterine position effects. *Neurosci Biobehav Rev* 26:665–678
 137. Muñoz-de-Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM 2005 Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. *Endocrinology* 146:4138–4147
 138. Wadia PR, Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM 2007 Perinatal bisphenol A exposure increases estrogen sensitivity of the mammary gland in diverse mouse strains. *Environ Health Perspect* 115:592–598
 139. Prins GS, Birch L, Tang WY, Ho SM 2007 Developmental estrogen exposures predispose to prostate carcinogenesis with aging. *Reprod Toxicol* 23:374–382
 140. Prins GS, Tang WY, Belmonte J, Ho SM 2008 Perinatal exposure to oestradiol and bisphenol A alters the prostate epigenome and increases susceptibility to carcinogenesis. *Basic Clin Pharmacol Toxicol* 102:134–138
 141. Prins GS, Ye SH, Birch L, Ho SM, Kannan K 2011 Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague-Dawley rats. *Reprod Toxicol* 31:1–9
 142. Bjørnerem A, Straume B, Midtby M, Fønnebo V, Sundsfjord J, Svartberg J, Acharya G, Oian P, Berntsen GK 2004 Endogenous sex hormones in relation to age, sex, lifestyle factors, and chronic diseases in a general population: the Tromsø Study. *J Clin Endocrinol Metab* 89:6039–6047
 143. Silva E, Rajapakse N, Kortenkamp A 2002 Something from “nothing”: eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. *Environ Sci Technol* 36:1751–1756
 144. Soto AM, Fernandez MF, Luizzi MF, Oles Karasko AS, Sonnenschein C 1997 Developing a marker of exposure to xenoestrogen mixtures in human serum. *Environ Health Perspect* 105:647–654
 145. Crofton KM 2008 Thyroid disrupting chemicals: mechanisms and mixtures. *Int J Androl* 31:209–223
 146. Montano MM, Welshons WV, vom Saal FS 1995 Free estradiol in serum and brain uptake of estradiol during fetal and neonatal sexual differentiation in female rats. *Biol Reprod* 53:1198–1207
 147. Nunez EA, Benassayag C, Savu L, Vallette G, Delorme J 1979 Oestrogen binding function of α 1-fetoprotein. *J Steroid Biochem* 11:237–243
 148. Milligan SR, Khan O, Nash M 1998 Competitive binding of xenobiotic oestrogens to rat α -fetoprotein and to sex steroid binding proteins in human and rainbow trout (*Oncorhynchus mykiss*) plasma. *Gen Comp Endocrinol* 112: 89–95
 149. Sheehan DM, Young M 1979 Diethylstilbestrol and estradiol binding to serum albumin and pregnancy plasma of rat and human. *Endocrinology* 104:1442–1446
 150. Déchaud H, Ravard C, Claustrat F, de la Perrière AB, Pugeat M 1999 Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). *Steroids* 64: 328–334
 151. Liu SV, Schally AV, Hawes D, Xiong S, Fazli L, Gleave M, Cai J, Groshen S, Brands F, Engel J, Pinski J 2010 Expression of receptors for luteinizing hormone-releasing hormone (LH-RH) in prostate cancers following therapy with LH-RH agonists. *Clin Cancer Res* 16:4675–4680
 152. Piccart M, Parker LM, Pritchard KI 2003 Oestrogen receptor downregulation: an opportunity for extending the window of endocrine therapy in advanced breast cancer. *Ann Oncol* 14:1017–1025
 153. Grandien K, Berkenstam A, Gustafsson JA 1997 The estrogen receptor gene: promoter organization and expression. *Int J Biochem Cell Biol* 29:1343–1369
 154. Morani A, Warner M, Gustafsson JA 2008 Biological functions and clinical implications of oestrogen receptors α and β in epithelial tissues. *J Intern Med* 264:128–142
 155. Mostaghel EA, Montgomery RB, Lin DW 2007 The basic biochemistry and molecular events of hormone therapy. *Curr Urol Rep* 8:224–232
 156. Phoenix CH, Goy RW, Gerall AA, Young WC 1959 Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology* 65:369–382
 157. Vom Saal FS, Moyer CL 1985 Prenatal effects on reproductive capacity during aging in female mice. *Biol Reprod* 32:1116–1126
 158. Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, Nadal A 2010 Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. *Environ Health Perspect* 118:1243–1250
 159. Even MD, Dhar MG, vom Saal FS 1992 Transport of steroids between fetuses via amniotic fluid in relation to the

- intrauterine position phenomenon in rats. *J Reprod Fertil* 96:709–716
160. vom Saal FS, Quadagno DM, Even MD, Keisler LW, Keisler DH, Khan S 1990 Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine positions. *Biol Reprod* 43:751–761
 161. vom Saal FS, Bronson FH 1978 *In utero* proximity of female mouse fetuses to males: effect on reproductive performance during later life. *Biol Reprod* 19:842–853
 162. Kinsley CH, Konen CM, Miele JL, Ghiraldi L, Svare B 1986 Intrauterine position modulates maternal behaviors in female mice. *Physiol Behav* 36:793–799
 163. Gandelman R, vom Saal FS, Reinisch JM 1977 Contiguity to male foetuses affects morphology and behaviour of female mice. *Nature* 266:722–724
 164. Palanza P, Parmigiani S, vom Saal FS 1995 Urine marking and maternal aggression of wild female mice in relation to anogenital distance at birth. *Physiol Behav* 58:827–835
 165. vom Saal FS, Grant WM, McMullen CW, Laves KS 1983 High fetal estrogen concentrations: correlation with increased adult sexual activity and decreased aggression in male mice. *Science* 220:1306–1309
 166. Palanza P, Morley-Fletcher S, Laviola G 2001 Novelty seeking in periadolescent mice: sex differences and influence of intrauterine position. *Physiol Behav* 72:255–262
 167. Clark MM, vom Saal FS, Galef Jr BG 1992 Intrauterine positions and testosterone levels of adult male gerbils are correlated. *Physiol Behav* 51:957–960
 168. vom Saal FS 1989 Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses *in utero*. *J Anim Sci* 67:1824–1840
 169. vom Saal FS 1989 The production of and sensitivity to cues that delay puberty and prolong subsequent oestrous cycles in female mice are influenced by prior intrauterine position. *J Reprod Fertil* 86:457–471
 170. Vom Saal FS, Even MD, Quadagno DM 1991 Effects of maternal stress on puberty, fertility and aggressive behavior of female mice from different intrauterine positions. *Physiol Behav* 49:1073–1078
 171. vom Saal FS, Pryor S, Bronson FH 1981 Effects of prior intrauterine position and housing on oestrous cycle length in adolescent mice. *Journal of Reproduction, Fertility* 62: 33–37
 172. Vandenberg JG, Huggett CL 1994 Mother's prior intrauterine position affects the sex ratio of her offspring in house mice. *Proc Natl Acad Sci USA* 91:11055–11059
 173. Vandenberg JG, Huggett CL 1995 The anogenital distance index, a predictor of the intrauterine position effects on reproduction in female house mice. *Lab Anim Sci* 45: 567–573
 174. Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenberg JG, vom Saal FS 1999 Exposure to bisphenol A advances puberty. *Nature* 401:763–764
 175. vom Saal FS, Bronson FH 1980 Variation in length of oestrous cycles in mice due to former intrauterine proximity to male fetuses. *Biol Reprod* 22:777–780
 176. Vandenberg LN, Maffini MV, Wadia PR, Sonnenschein C, Rubin BS, Soto AM 2007 Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. *Endocrinology* 148:116–127
 177. Timms BG, Petersen SL, vom Saal FS 1999 Prostate gland growth during development is stimulated in both male and female rat fetuses by intrauterine proximity to female fetuses. *J Urol* 161:1694–1701
 178. Nonneman DJ, Ganjam VK, Welshons WV, Vom Saal FS 1992 Intrauterine position effects on steroid metabolism and steroid receptors of reproductive organs in male mice. *Biol Reprod* 47:723–729
 179. Clark MM, Bishop AM, vom Saal FS, Galef Jr BG 1993 Responsiveness to testosterone of male gerbils from known intrauterine positions. *Physiol Behav* 53:1183–1187
 180. vom Saal FS, Bronson FH 1980 Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. *Science* 208: 597–599
 181. Timms BG, Peterson RE, vom Saal FS 2002 2,3,7,8-tetrachlorodibenzo-*p*-dioxin interacts with endogenous estradiol to disrupt prostate gland morphogenesis in male rat fetuses. *Toxicol Sci* 67:264–274
 182. Vandenberg JG 2004 Animal models and studies of *in utero* endocrine disruptor effects. *ILAR J* 45:438–442
 183. Clark MM, Crews D, Galef Jr BG 1991 Concentrations of sex steroid hormones in pregnant and fetal Mongolian gerbils. *Physiol Behav* 49:239–243
 184. Satoh S, Hirata T, Miyake Y, Kaneda Y 1997 The possibility of early estimation for fertility in bovine heterosexual twin females. *J Vet Med Sci* 59:221–222
 185. Padula AM 2005 The freemartin syndrome: an update. *Anim Reprod Sci* 87:93–109
 186. Resnick SM, Gottesman II, McGue M 1993 Sensation seeking in opposite-sex twins: an effect of prenatal hormones? *Behav Genet* 23:323–329
 187. McFadden D 1993 A masculinizing effect on the auditory systems of human females having male co-twins. *Proc Natl Acad Sci USA* 90:11900–11904
 188. Cohen-Bendahan CC, Buitelaar JK, van Goozen SH, Cohen-Kettenis PT 2004 Prenatal exposure to testosterone and functional cerebral lateralization: a study in same-sex and opposite-sex twin girls. *Psychoneuroendocrinology* 29:911–916
 189. Peper JS, Brouwer RM, van Baal GC, Schnack HG, van Leeuwen M, Boomsma DI, Kahn RS, Hulshoff Pol HE 2009 Does having a twin brother make for a bigger brain? *Eur J Endocrinol* 160:739–746
 190. Cohen-Bendahan CC, Buitelaar JK, van Goozen SH, Orlebeke JF, Cohen-Kettenis PT 2005 Is there an effect of prenatal testosterone on aggression and other behavioral traits? A study comparing same-sex and opposite-sex twin girls. *Horm Behav* 47:230–237
 191. Loehlin JC, Martin NG 2000 Dimensions of psychological masculinity-femininity in adult twins from opposite-sex and same-sex pairs. *Behav Genet* 30:19–28
 192. Rose RJ, Kaprio J, Winter T, Dick DM, Viken RJ, Pulkkinen L, Koskenvuo M 2002 Femininity and fertility in sisters with twin brothers: prenatal androgenization? Cross-sex socialization? *Psychol Sci* 13:263–267
 193. Vuoksimaa E, Eriksson CJ, Pulkkinen L, Rose RJ, Kaprio J 2010 Decreased prevalence of left-handedness among females with male co-twins: evidence suggesting prenatal tes-

- tosterone transfer in humans? *Psychoneuroendocrinology* 35:1462–1472
194. Elkadi S, Nicholls ME, Clode D 1999 Handedness in opposite and same-sex dizygotic twins: testing the testosterone hypothesis. *Neuroreport* 10:333–336
195. Lummaa V, Pettay JE, Russell AF 2007 Male twins reduce fitness of female co-twins in humans. *Proc Natl Acad Sci USA* 104:10915–10920
196. van Anders SM, Vernon PA, Wilbur CJ 2006 Finger-length ratios show evidence of prenatal hormone-transfer between opposite-sex twins. *Horm Behav* 49:315–319
197. Culbert KM, Breedlove SM, Burt SA, Klump KL 2008 Prenatal hormone exposure and risk for eating disorders. *Arch Gen Psychiatry* 65:329–336
198. Glinianaia SV, Magnus P, Harris JR, Tams K 1998 Is there a consequence for fetal growth of having an unlike-sexed cohabitant *in utero*? *Int J Epidemiol* 27:657–659
199. Cerhan JR, Kushi LH, Olson JE, Rich SS, Zheng W, Folsom AR, Sellers TA 2000 Twinship and risk of postmenopausal breast cancer. *J Natl Cancer Inst* 92:261–265
200. Swerdlow AJ, De Stavola BL, Swanwick MA, Maconochie NES 1997 Risks of breast and testicular cancers in young adult twins in England and Wales: evidence on prenatal and genetic aetiology. *Lancet* 350:1723–1728
201. van de Beek C, Thijssen JH, Cohen-Kettenis PT, van Goozen SH, Buitelaar JK 2004 Relationships between sex hormones assessed in amniotic fluid, and maternal and umbilical cord serum: what is the best source of information to investigate the effects of fetal hormone exposure? *Horm Behav* 46:663–669
202. Sakai LM, Baker LA, Jacklin CN, Shulman I 1991 Sex steroids at birth: genetic and environmental variation and covariation. *Dev Psychobiol* 24:559–570
203. Cohen-Bendahan CC, van Goozen SH, Buitelaar JK, Cohen-Kettenis PT 2005 Maternal serum steroid levels are unrelated to fetal sex: a study in twin pregnancies. *Twin Res Hum Genet* 8:173–177
204. Johnson MR, Abbas A, Nicolaidis KH 1994 Maternal plasma levels of human chorionic gonadotropin, oestradiol and progesterone in multifetal pregnancies before and after fetal reduction. *J Endocrinol* 143:309–312
205. Vom Saal FS, Richter CA, Ruhlen RR, Nagel SC, Timms BG, Welshons WV 2005 The importance of appropriate controls, animal feed, and animal models in interpreting results from low-dose studies of bisphenol A. *Birth Defects Res A Clin Mol Teratol* 73:140–145
206. Spearow JL, Doemeny P, Sera R, Leffler R, Barkley M 1999 Genetic variation in susceptibility to endocrine disruption by estrogen in mice. *Science* 285:1259–1261
207. Spearow JL, O’Henley P, Doemeny P, Sera R, Leffler R, Sofos T, Barkley M 2001 Genetic variation in physiological sensitivity to estrogen in mice. *APMIS* 109:356–364
208. Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS 2005 Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. *Proc Natl Acad Sci USA* 102:7014–7019
209. Cederroth CR, Nef S 2009 Fetal programming of adult glucose homeostasis in mice. *PLoS ONE* 4:e7281
210. Marty MS, Carney EW, Rowlands JC 2011 Endocrine disruption: historical perspectives and its impact on the future of toxicology testing. *Toxicol Sci* 120:S93–S108
211. Bonefeld-Jørgensen EC, Long M, Hofmeister MV, Vinggaard AM 2007 Endocrine-disrupting potential of bisphenol A, bisphenol A dimethacrylate, 4-n-nonylphenol, and 4-n-octylphenol in vitro: new data and a brief review. *Environ Health Perspect* 115(Suppl 1):69–76
212. Krüger T, Long M, Bonefeld-Jørgensen EC 2008 Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor. *Toxicology* 246:112–123
213. Watson CS, Jeng YJ, Kochukov MY 2010 Nongenomic signaling pathways of estrogen toxicity. *Toxicol Sci* 115:1–11
214. Weed DL 2005 Weight of evidence: a review of concepts and methods. *Risk Anal* 25:1545–1557
215. Linkov I, Loney D, Cormier S, Satterstrom FK, Bridges T 2009 Weight-of-evidence evaluation in environmental assessment: review of qualitative and quantitative approaches. *Sci Total Environ* 407:5199–5205
216. Schreider J, Barrow C, Birchfield N, Dearfield K, Devlin D, Henry S, Kramer M, Schappelle S, Solomon K, Weed DL, Embry MR 2010 Enhancing the credibility of decisions based on scientific conclusions: transparency is imperative. *Toxicol Sci* 116:5–7
217. Basketter D, Ball N, Cagen S, Carrillo JC, Certa H, Eigler D, Garcia C, Esch H, Graham C, Haux C, Kreiling R, Mehling A 2009 Application of a weight of evidence approach to assessing discordant sensitisation datasets: implications for REACH. *Regul Toxicol Pharmacol* 55:90–96
218. Wright-Walters M, Volz C, Talbott E, Davis D 2011 An updated weight of evidence approach to the aquatic hazard assessment of bisphenol A and the derivation a new predicted no effect concentration (Pnec) using a non-parametric methodology. *Sci Total Environ* 409:676–685
219. Cooper RL, Kavlock RJ 1997 Endocrine disruptors and reproductive development: a weight-of-evidence overview. *J Endocrinol* 152:159–166
220. Popp JA, Crouch E, McConnell EE 2006 A weight-of-evidence analysis of the cancer dose-response characteristics of 2,3,7,8-tetrachlorodibenzodioxin (TCDD). *Toxicol Sci* 89:361–369
221. Goodman M, Squibb K, Youngstrom E, Anthony LG, Kenworthy L, Lipkin PH, Mattison DR, Lakind JS 2010 Using systematic reviews and meta-analyses to support regulatory decision making for neurotoxicants: lessons learned from a case study of PCBs. *Environ Health Perspect* 118:727–734
222. Goodman JE, Witorsch RJ, McConnell EE, Sipes IG, Slayton TM, Yu CJ, Franz AM, Rhomberg LR 2009 Weight-of-evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. *Crit Rev Toxicol* 39:1–75
223. Heindel JJ, vom Saal FS 2008 Meeting report: batch-to-batch variability in estrogenic activity in commercial animal diets- importance and approaches for laboratory animal research. *Environ Health Perspect* 116:389–393
224. Ruhlen RL, Taylor JA, Mao J, Kirkpatrick J, Welshons WV, vom Saal FS 2011 Choice of animal feed can alter fetal steroid levels and mask developmental effects of endocrine disrupting chemicals. *J Dev Origins Health Dis* 2:36–48

225. vom Saal FS, Richter CA, Mao J, Welshons WV 2005 Commercial animal feed: variability in estrogenic activity and effects on body weight in mice. *Birth Defects Res (Part A)* 73:474–475
226. Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orzario CE, Ruhlen RL, Vom Saal FS, Welshons WV 2003 Bisphenol A is released from polycarbonate animal cages into water at room temperature. *Environ Health Perspect* 111:1180–1187
227. Koehler KE, Voigt RC, Thomas S, Lamb B, Urban C, Hassold T, Hunt PA 2003 When disaster strikes: rethinking caging materials. *Lab Anim (NY)* 32:24–27
228. Muhlhauser A, Susiarjo M, Rubio C, Griswold J, Gorence G, Hassold T, Hunt PA 2009 Bisphenol A effects on the growing mouse oocyte are influenced by diet. *Biol Reprod* 80:1066–1071
229. Tyl RW, Myers CB, Marr MC, Castillo NP, Veselica MM, Joiner RL, Dimond SS, Van Miller JP, Stropp GD, Waechter Jr JM, Hentges SG 2008 One-generation reproductive toxicity study of dietary 17 β -estradiol (E2; CAS no. 50-28-2) in CD-1 (Swiss) mice. *Reprod Toxicol* 25:144–160
230. Ryan BC, Hotchkiss AK, Crofton KM, Gray Jr LE 2010 *In utero* and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. *Toxicol Sci* 114:133–148
231. Marty MS, Allen B, Chapin RE, Cooper R, Daston GP, Flaws JA, Foster PM, Makris SL, Mylchreest E, Sandler D, Tyl RW 2009 Inter-laboratory control data for reproductive endpoints required in the OPPTS 870.3800/OECD 416 reproduction and fertility test. *Birth Defects Res B Dev Reprod Toxicol* 86:470–489
232. Teng CT, Beard C, Gladwell W 2002 Differential expression and estrogen response of lactoferrin gene in the female reproductive tract of mouse, rat, and hamster. *Biol Reprod* 67:1439–1449
233. Aupperlee MD, Drolet AA, Durairaj S, Wang W, Schwartz RC, Haslam SZ 2009 Strain-specific differences in the mechanisms of progesterone regulation of murine mammary gland development. *Endocrinology* 150:1485–1494
234. Pepling ME, Sundman EA, Patterson NL, Gephardt GW, Medico L Jr, Wilson KI 2010 Differences in oocyte development and estradiol sensitivity among mouse strains. *Reproduction* 139:349–357
235. Wiklund JA, Gorski J 1982 Genetic differences in estrogen-induced DNA synthesis in the rat pituitary: correlations with pituitary tumor susceptibility. *Endocrinology* 111:1140–1149
236. Wiklund J, Wertz N, Gorski J 1981 A comparison of estrogen effects on uterine and pituitary growth and prolactin synthesis in F344 and Holtzman rats. *Endocrinology* 109:1700–1707
237. Diel P, Schmidt S, Vollmer G, Janning P, Upmeyer A, Michna H, Bolt HM, Degen GH 2004 Comparative responses of three rat strains (DA/Han, Sprague-Dawley and Wistar) to treatment with environmental estrogens. *Arch Toxicol* 78:183–193
238. Brossia LJ, Roberts CS, Lopez JT, Bigsby RM, Dynlacht JR 2009 Interstrain differences in the development of pyometra after estrogen treatment of rats. *J Am Assoc Lab Anim Sci* 48:517–520
239. Geis RB, Diel P, Degen GH, Vollmer G 2005 Effects of genistein on the expression of hepatic genes in two rat strains (Sprague-Dawley and Wistar). *Toxicol Lett* 157:21–29
240. Roper RJ, Griffith JS, Lyttle CR, Doerge RW, McNabb AW, Broadbent RE, Teuscher C 1999 Interacting quantitative trait loci control phenotypic variation in murine estradiol-regulated responses. *Endocrinology* 140:556–561
241. Taylor JA, Welshons WV, Vom Saal FS 2008 No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24h after administration in neonatal female mice. *Reprod Toxicol* 25:169–176
242. European Food Safety Authority 2007 Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2,2-bis(4-hydroxyphenyl)propane. *EFSA J* 428:1–75
243. Vandenberg LN, Chahoud I, Padmanabhan V, Paumgarten FJ, Schoenfelder G 2010 Biomonitoring studies should be used by regulatory agencies to assess human exposure levels and safety of bisphenol A. *Environ Health Perspect* 118:1051–1054
244. Vandenberg LN 2011 Exposure to bisphenol A in Canada: invoking the precautionary principle. *CMAJ* 183:1265–1270
245. Stahlhut RW, Welshons WV, Swan SH 2009 Bisphenol A data in NHANES suggest longer than expected half-life, substantial non-food exposure, or both. *Environ Health Perspect* 117:784–789
246. Geens T, Goeyens L, Covaci A 2011 Are potential sources for human exposure to bisphenol-A overlooked? *Int J Hyg Environ Health* 214:339–347
247. Biedermann S, Tschudin P, Grob K 2010 Transfer of bisphenol A from thermal printer paper to the skin. *Anal Bioanal Chem* 398:571–576
248. Zalko D, Jacques C, Duplan H, Bruel S, Perdu E 2011 Viable skin efficiently absorbs and metabolizes bisphenol A. *Chemosphere* 82:424–430
249. Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, Kanamoto N, Hataya Y, Shimatsu A, Kuzuya H, Nakao K 2002 Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J Clin Endocrinol Metab* 87:5185–5190
250. Zoeller RT, Bansal R, Parris C 2005 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* 146:607–612
251. Lee HJ, Chattopadhyay S, Gong EY, Ahn RS, Lee K 2003 Antiandrogenic effects of bisphenol A and nonphenol on the function of androgen receptor. *Toxicol Sci* 75:40–46
252. Kwintkiewicz J, Nishi Y, Yanase T, Giudice LC 2010 Peroxisome proliferator-activated receptor- γ mediates bisphenol A inhibition of FSH-stimulated IGF-1, aromatase, and estradiol in human granulosa cells. *Environ Health Perspect* 118:400–406
253. Taylor JA, Vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, Toutain PL, Laffont CM, Vandervoort CA 2011 Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. *Environ Health Perspect* 119:422–430
254. Owens JW, Chaney JG 2005 Weighing the results of differing ‘low dose’ studies of the mouse prostate by

- Nagel, Cagen, and Ashby: quantification of experimental power and statistical results. *Regul Toxicol Pharmacol* 43:194–202
255. Ashby J, Tinwell H, Odum J, Lefevre P 2004 Natural variability and the influence of concurrent control values on the detection and interpretation of low-dose or weak endocrine toxicities. *Environ Health Perspect* 112:847–853
256. Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV 1997 Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens bisphenol A and octylphenol. *Environ Health Perspect* 105:70–76
257. Gupta C 2000 Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. *Proc Soc Exp Biol Med* 224:61–68
258. Elswick BA, Welsch F, Janszen DB 2000 Effect of different sampling designs on outcome of endocrine disruptor studies. *Reprod Toxicol* 14:359–367
259. Chitra KC, Latchoumycandane C, Mathur PP 2003 Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. *Toxicology* 185:119–127
260. Ramos JG, Varayoud J, Sonnenschein C, Soto AM, Muñoz De Toro M, Luque EH 2001 Prenatal exposure to low doses of bisphenol A alters the periductal stroma and glandular cell function in the rat ventral prostate. *Biol Reprod* 65:1271–1277
261. Ramos JG, Varayoud J, Kass L, Rodríguez H, Costabel L, Muñoz-De-Toro M, Luque EH 2003 Bisphenol A induces both transient and permanent histofunctional alterations of the hypothalamic-pituitary-gonadal axis in prenatally exposed male rats. *Endocrinology* 144:3206–3215
262. Ogura Y, Ishii K, Kanda H, Kanai M, Arima K, Wang Y, Sugimura Y 2007 Bisphenol A induces permanent squamous change in mouse prostatic epithelium. *Differentiation* 75:745–756
263. Ho SM, Tang WY, Belmonte de Frausto J, Prins GS 2006 Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res* 66:5624–5632
264. Ichihara T, Yoshino H, Imai N, Tsutsumi T, Kawabe M, Tamano S, Inaguma S, Suzuki S, Shirai T 2003 Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. *J Toxicol Sci* 28:165–171
265. Ashby J, Tinwell H, Haseman J 1999 Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed *in utero*. *Regul Toxicol Pharmacol* 30:156–166
266. Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR 1999 Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. *Toxicol Sci* 50:36–44
267. Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR 1999 Normal reproductive organ development in Wistar rats exposed to bisphenol A in the drinking water. *Regul Toxicol Pharmacol* 30:130–139
268. Ema M, Fujii S, Furukawa M, Kiguchi M, Harazono A 2001 Rat two-generation reproductive toxicity study of bisphenol A. *Reprod Toxicol* 15:505–523
269. Tinwell H, Haseman J, Lefevre PA, Wallis N, Ashby J 2002 Normal sexual development of two strains of rat exposed *in utero* to low doses of bisphenol A. *Toxicol Sci* 68:339–348
270. Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, Waechter JM 2002 Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. *Toxicol Sci* 68:121–146
271. Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter Jr JM 2008 Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. *Toxicol Sci* 104:362–384
272. Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, Gray Jr LE 2008 Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat. *Toxicol Sci* 102:371–382
273. Chapin RE, Adams J, Boekelheide K, Gray LE Jr, Hayward SW, Lees PS, McIntyre BS, Portier KM, Schnorr TM, Selivan SG, Vandenberg JG, Woskie SR 2008 NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. *Birth Defects Res B Dev Reprod Toxicol* 83:157–395
274. Hennighausen L, Robinson GW 1998 Think globally, act locally: the making of a mouse mammary gland. *Genes Dev* 12:449–455
275. Lemmen JG, Broekhof JL, Kuiper GG, Gustafsson JA, van der Saag PT, van der Burg B 1999 Expression of estrogen receptor α and β during mouse embryogenesis. *Mech Dev* 81:163–167
276. Padilla-Banks E, Jefferson WN, Newbold RR 2006 Neonatal exposure to the phytoestrogen genistein alters mammary gland growth and developmental programming of hormone receptor levels. *Endocrinology* 147:4871–4882
277. Colerangle JB, Roy D 1997 Profound effects of the weak environmental estrogen-like chemical bisphenol A on the growth of the mammary gland of Noble rats. *J Steroid Biochem Mol Biol* 60:153–160
278. Bern HA, Mills KT, Jones LA 1983 Critical period of neonatal estrogen exposure in occurrence of mammary gland abnormalities in adult mice. *Proc Soc Exp Biol Med* 172:239–242
279. Markey CM, Coombs MA, Sonnenschein C, Soto AM 2003 Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. *Evol Dev* 5:67–75
280. Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C, Soto AM 2001 *In utero* exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod* 65:1215–1223
281. Vandenberg LN, Maffini MV, Schaeberle CM, Ucci AA, Sonnenschein C, Rubin BS, Soto AM 2008 Perinatal exposure to the xenoestrogen bisphenol-A induces mammary

- intraductal hyperplasias in adult CD-1 mice. *Reprod Toxicol* 26:210–219
282. Moral R, Wang R, Russo IH, Lamartiniere CA, Pereira J, Russo J 2008 Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. *J Endocrinol* 196:101–112
 283. Ayyanan A, Laribi O, Schuepbach-Malpell S, Schrick C, Gutierrez M, Tanos T, Lefebvre G, Rougemont J, Yalcin-Ozuysal O, Brisken C 2011 Perinatal exposure to bisphenol A increases adult mammary gland progesterone response and cell number. *Mol Endocrinol* 25:1915–1923
 284. Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A 2004 Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. *Reprod Toxicol* 18:803–811
 285. Jones LP, Sampson A, Kang HJ, Kim HJ, Yi YW, Kwon SY, Babus JK, Wang A, Bae I 2010 Loss of BRCA1 leads to an increased sensitivity to bisphenol A. *Toxicol Lett* 199:261–268
 286. Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM 2007 Induction of mammary gland ductal hyperplasias and carcinomas in situ following fetal bisphenol A exposure. *Reprod Toxicol* 23:383–390
 287. Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Luque EH, Muñoz-de-Toro M 2007 Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. *Environ Health Perspect* 115:80–86
 288. Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J, Lamartiniere CA 2009 Oral exposure to bisphenol A increases dimethylbenzanthracene-induced mammary cancer in rats. *Environ Health Perspect* 117:910–915
 289. Betancourt AM, Eltoum IA, Desmond RA, Russo J, Lamartiniere CA 2010 *In utero* exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat. *Environ Health Perspect* 118:1614–1619
 290. Weber Lozada K, Keri RA 2011 Bisphenol A increases mammary cancer risk in two distinct mouse models of breast cancer. *Biol Reprod* 85:490–497
 291. Betancourt AM, Mobley JA, Russo J, Lamartiniere CA 2010 Proteomic analysis in mammary glands of rat offspring exposed *in utero* to bisphenol A. *J Proteomics* 73:1241–1253
 292. Lamartiniere CA, Jenkins S, Betancourt AM, Wang J, Russo J 2011 Exposure to the endocrine disruptor bisphenol A alters susceptibility for mammary cancer. *Horm Mol Biol Clin Investig* 5:45–52
 293. Jenkins S, Wang J, Eltoum I, Desmond R, Lamartiniere CA 2011 Chronic oral exposure to bisphenol A results in a non-monotonic dose response in mammary carcinogenesis and metastasis in MMTV-erbB2 mice. *Environ Health Perspect* 119:1604–1609
 294. Nikaido Y, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A 2005 Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice. *In Vivo* 19:487–494
 295. Yin H, Ito A, Bhattacharjee D, Hoshi M 2006 A comparative study on the protective effects of 17 β -estradiol, biochanin A and bisphenol A on mammary gland differentiation and tumorigenesis in rats. *Indian J Exp Biol* 44:540–546
 296. Yang M, Ryu JH, Jeon R, Kang D, Yoo KY 2009 Effects of bisphenol A on breast cancer and its risk factors. *Arch Toxicol* 83:281–285
 297. Kortenkamp A 2006 Breast cancer, oestrogens and environmental pollutants: a re-evaluation from a mixture perspective. *Int J Androl* 29:193–198
 298. Hunt PA, Susiarjo M, Rubio C, Hassold TJ 2009 The bisphenol A experience: a primer for the analysis of environmental effects on mammalian reproduction. *Biol Reprod* 81:807–813
 299. Carr R, Bertasi F, Betancourt A, Bowers S, Gandy BS, Ryan P, Willard S 2003 Effect of neonatal rat bisphenol A exposure on performance in the Morris water maze. *J Toxicol Environ Health A* 66:2077–2088
 300. Farabollini F, Porrini S, Dessì-Fulgherit F 1999 Perinatal exposure to the estrogenic pollutant bisphenol A affects behavior in male and female rats. *Pharmacol Biochem Behav* 64:687–694
 301. Fujimoto T, Kubo K, Aou S 2006 Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. *Brain Res* 1068:49–55
 302. Funabashi T, Kawaguchi M, Furuta M, Fukushima A, Kimura F 2004 Exposure to bisphenol A during gestation and lactation causes loss of sex difference in corticotropin-releasing hormone-immunoreactive neurons in the bed nucleus of the stria terminalis of rats. *Psychoneuroendocrinology* 29:475–485
 303. Kubo K, Arai O, Omura M, Watanabe R, Ogata R, Aou S 2003 Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. *Neurosci Res* 45:345–356
 304. Kubo K, Arai O, Ogata R, Omura M, Hori T, Aou S 2001 Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behaviour in the rat. *Neurosci Lett* 304:73–76
 305. Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM 2006 Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. *Endocrinology* 147:3681–3691
 306. Patisaul HB, Fortino AE, Polston EK 2006 Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. *Neurotoxicol Teratol* 28:111–118
 307. Adewale HB, Todd KL, Mickens JA, Patisaul HB 2011 The impact of neonatal bisphenol: a exposure on sexually dimorphic hypothalamic nuclei in the female rat. *Neurotoxicology* 32:38–49
 308. Wolstenholme JT, Rissman EF, Connelly JJ 2011 The role of bisphenol A in shaping the brain, epigenome and behavior. *Horm Behav* 59:296–305
 309. Maffini MV, Rubin BS, Sonnenschein C, Soto AM 2006 Endocrine disruptors and reproductive health: the case of bisphenol-A. *Mol Cell Endocrinol* 254–255:179–186
 310. Markey CM, Wadia PR, Rubin BS, Sonnenschein C, Soto AM 2005 Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. *Biol Reprod* 72:1344–1351
 311. Yoshino S, Yamaki K, Li X, Sai T, Yanagisawa R, Takano H, Taneda S, Hayashi H, Mori Y 2004 Prenatal exposure to bisphenol A up-regulates immune responses, including

- T helper 1 and T helper 2 responses, in mice. *Immunology* 112:489–495
312. Yoshino S, Yamaki K, Yanagisawa R, Takano H, Hayashi H, Mori Y 2003 Effects of bisphenol A on antigen-specific antibody production, proliferative responses of lymphoid cells, and TH1 and TH2 immune responses in mice. *Br J Pharmacol* 138:1271–1276
313. Alonso-Magdalena P, Ropero AB, Soriano S, Quesada I, Nadal A 2010 Bisphenol-A: a new diabetogenic factor? *Hormones (Athens)* 9:118–126
314. Rubin BS, Soto AM 2009 Bisphenol A: perinatal exposure and body weight. *Mol Cell Endocrinol* 304:55–62
315. Al-Hiyasat AS, Darmani H, Elbetieha AM 2002 Effects of bisphenol A on adult male mouse fertility. *Eur J Oral Sci* 110:163–167
316. Cabaton NJ, Wadia PR, Rubin BS, Zalko D, Schaeberle CM, Askenase MH, Gadbois JL, Tharp AP, Whitt GS, Sonnenschein C, Soto AM 2011 Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice. *Environ Health Perspect* 119:547–552
317. Al-Hiyasat AS, Darmani H, Elbetieha AM 2004 Leached components from dental composites and their effects on fertility of female mice. *Eur J Oral Sci* 112:267–272
318. Salian S, Doshi T, Vanage G 2009 Impairment in protein expression profile of testicular steroid receptor coregulators in male rat offspring perinatally exposed to Bisphenol A. *Life Sci* 85:11–18
319. Rubin BS 2011 Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. *J Steroid Biochem Mol Biol* 127:27–34
320. Battaglin WA, Rice KC, Focazio MJ, Salmons S, Barry RX 2009 The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005–2006. *Environ Monit Assess* 155:281–307
321. Battaglin WA, Furlong ET, Burkhardt MR, Peter CJ 2000 Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, 1998. *Sci Total Environ* 248:123–133
322. Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TM, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall Jr LW, Williams W 1996 Ecological risk assessment of atrazine in North American surface waters. *Environ Toxicol Chem* 15:31–76
323. Benachour N, Moslemi S, Sipahutar H, Seralini GE 2007 Cytotoxic effects and aromatase inhibition by xenobiotic endocrine disruptors alone and in combination. *Toxicol Appl Pharmacol* 222:129–140
324. Sanderson JT, Seinen W, Giesy JP, van den Berg M 2000 2-Chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity? *Toxicol Sci* 54:121–127
325. Sanderson JT, Letcher RJ, Heneweer M, Giesy JP, van den Berg M 2001 Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. *Environ Health Perspect* 109:1027–1031
326. Hayes TB, Anderson LL, Beasley VR, de Solla SR, Iguchi T, Ingraham H, Kestemont P, Kniewald J, Kniewald Z, Langlois VS, Luque EH, McCoy KA, Muñoz-de-Toro M, Oka T, Oliveira CA, Orton F, Ruby S, Suzawa M, Tavera-Mendoza LE, Trudeau VL, Victor-Costa AB, Willingham E 2011 Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. *J Steroid Biochem Mol Biol* 127:64–73
327. Cooper RL, Laws SC, Das PC, Narotsky MG, Goldman JM, Lee Tyrey E, Stoker TE 2007 Atrazine and reproductive function: mode and mechanism of action studies. *Birth Defects Res B Dev Reprod Toxicol* 80:98–112
328. Stoker TE, Robinette CL, Cooper RL 1999 Maternal exposure to atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring. *Toxicol Sci* 52:68–79
329. Laws SC, Hotchkiss M, Ferrell J, Jayaraman S, Mills L, Modic W, Tinfo N, Fraites M, Stoker T, Cooper R 2009 Chlorotriazine herbicides and metabolites activate an ACTH-dependent release of corticosterone in male Wistar rats. *Toxicol Sci* 112:78–87
330. Fraites MJ, Cooper RL, Buckalew A, Jayaraman S, Mills L, Laws SC 2009 Characterization of the hypothalamic-pituitary-adrenal axis response to atrazine and metabolites in the female rat. *Toxicol Sci* 112:88–99
331. Yoshimoto S, Okada E, Umemoto H, Tamura K, Uno Y, Nishida-Umehara C, Matsuda Y, Takamatsu N, Shiba T, Ito M 2008 A W-linked DM-domain gene, DM-W, participates in primary ovary development in *Xenopus laevis*. *Proc Natl Acad Sci USA* 105:2469–2474
332. Hayes TB 1998 Sex determination and primary sex differentiation in amphibians. *J Exp Zool* 281:373–399
333. Ochoa-Acuña H, Frankenberger J, Hahn L, Carbajo C 2009 Drinking-water herbicide exposure in Indiana and prevalence of small-for-gestational-age and preterm delivery. *Environ Health Perspect* 117:1619–1624
334. Morgan MK, Scheuerman PR, Bishop CS, Pyles RA 1996 Teratogenic potential of atrazine and 2,4-D using FETAX. *J Toxicol Environ Health* 48:151–168
335. Allran JW, Karasov WH 2001 Effects of atrazine on embryos, larvae, and adults of anuran amphibians. *Environ Toxicol Chem* 20:769–775
336. Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, Vonk A 2002 Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proc Natl Acad Sci USA* 99:5476–5480
337. Hayes TB, Khoury V, Narayan A, Nazir M, Park A, Brown T, Adame L, Chan E, Buchholz D, Stueve T, Gallipeau S 2010 Atrazine induces complete feminization and chemical castration in male African clawed frogs (*Xenopus laevis*). *Proc Natl Acad Sci USA* 107:4612–4617
338. Hayes TB, Stuart AA, Mendoza M, Collins A, Noriega N, Vonk A, Johnston G, Liu R, Kpodzo D 2006 Characterization of atrazine-induced gonadal malformations in African clawed frogs (*Xenopus laevis*) and comparisons with effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (17 β -estradiol): support for the demasculinization/feminization hypothesis. *Environ Health Perspect* 114:134–141
339. Storrs-Méndez SI, Semlitsch RD 2010 Intersex gonads in frogs: understanding the time course of natural develop-

- ment and role of endocrine disruptors. *J Exp Zool B Mol Dev Evol* 314:57–66
340. Carr JA, Gentles A, Smith EE, Goleman WL, Urquidí LJ, Thuet K, Kendall RJ, Giesy JP, Gross TS, Solomon KR, Van Der Kraak G 2003 Response of larval *Xenopus laevis* to atrazine: assessment of growth, metamorphosis, and gonadal and laryngeal morphology. *Environ Toxicol Chem* 22:396–405
 341. Hecker M, Kim WJ, Park JW, Murphy MB, Villeneuve D, Coady KK, Jones PD, Solomon KR, Van Der Kraak G, Carr JA, Smith EE, du Preez L, Kendall RJ, Giesy JP 2005 Plasma concentrations of estradiol and testosterone, gonadal aromatase activity and ultrastructure of the testis in *Xenopus laevis* exposed to estradiol or atrazine. *Aquat Toxicol* 72:383–396
 342. Orton F, Carr JA, Handy RD 2006 Effects of nitrate and atrazine on larval development and sexual differentiation in the northern leopard frog *Rana pipiens*. *Environ Toxicol Chem* 25:65–71
 343. Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A 2003 Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. *Environ Health Perspect* 111:568–575
 344. Tavera-Mendoza L, Ruby S, Brousseau P, Fournier M, Cyr D, Marcogliese D 2002 Response of the amphibian tadpole (*Xenopus laevis*) to atrazine during sexual differentiation of the testis. *Environ Toxicol Chem* 21:527–531
 345. Oka T, Tooi O, Mitsui N, Miyahara M, Ohnishi Y, Takase M, Kashiwagi A, Shinkai T, Santo N, Iguchi T 2008 Effect of atrazine on metamorphosis and sexual differentiation in *Xenopus laevis*. *Aquat Toxicol* 87:215–226
 346. Langlois VS, Carew AC, Pauli BD, Wade MG, Cooke GM, Trudeau VL 2010 Low levels of the herbicide atrazine alter sex ratios and reduce metamorphic success in *Rana pipiens* tadpoles raised in outdoor mesocosms. *Environ Health Perspect* 118:552–557
 347. Jooste AM, Du Preez LH, Carr JA, Giesy JP, Gross TS, Kendall RJ, Smith EE, Van der Kraak GL, Solomon KR 2005 Gonadal development of larval male *Xenopus laevis* exposed to atrazine in outdoor microcosms. *Environ Sci Technol* 39:5255–5261
 348. Spolyarich N, Hyne R, Wilson S, Palmer C, Byrne M 2010 Growth, development and sex ratios of spotted marsh frog (*Limnodynastes tasmaniensis*) larvae exposed to atrazine and a herbicide mixture. *Chemosphere* 78:807–813
 349. Hecker M, Park JW, Murphy MB, Jones PD, Solomon KR, Van Der Kraak G, Carr JA, Smith EE, du Preez L, Kendall RJ, Giesy JP 2005 Effects of atrazine on CYP19 gene expression and aromatase activity in testes and on plasma sex steroid concentrations of male African clawed frogs (*Xenopus laevis*). *Toxicol Sci* 86:273–280
 350. Du Preez LH, Kunene N, Everson GJ, Carr JA, Giesy JP, Gross TS, Hosmer AJ, Kendall RJ, Smith EE, Solomon KR, Van Der Kraak GJ 2008 Reproduction, larval growth, and reproductive development in African clawed frogs (*Xenopus laevis*) exposed to atrazine. *Chemosphere* 71:546–552
 351. Kloas W, Lutz I, Springer T, Krueger H, Wolf J, Holden L, Hosmer A 2009 Does atrazine influence larval development and sexual differentiation in *Xenopus laevis*? *Toxicol Sci* 107:376–384
 352. U.S. Environmental Protection Agency 2010 October 9–12, 2007: The potential for atrazine to affect amphibian gonadal development. FIFRA Scientific Advisory Panel Meeting, Arlington, VA, 2007
 353. McDaniel TV, Martin PA, Struger J, Sherry J, Marvin CH, McMaster ME, Clarence S, Tetreault G 2008 Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) from areas of intensive row crop agriculture. *Aquat Toxicol* 88:230–242
 354. Reeder AL, Foley GL, Nichols DK, Hansen LG, Wikoff B, Faeh S, Eisold J, Wheeler MB, Warner R, Murphy JE, Beasley VR 1998 Forms and prevalence of intersexuality and effects of environmental contaminants on sexuality in cricket frogs (*Acris crepitans*). *Environ Health Perspect* 106:261–266
 355. Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A 2002 Feminization of male frogs in the wild. *Nature* 419:895–896
 356. Spolyarich N, Hyne RV, Wilson SP, Palmer CG, Byrne M 2011 Morphological abnormalities in frogs from a rice-growing region in NSW, Australia, with investigations into pesticide exposure. *Environ Monit Assess* 173:397–407
 357. Du Preez LH, Kunene N, Hanner R, Giesy JP, Solomon KR, Hosmer A, Van Der Kraak GJ 2009 Population-specific incidence of testicular ovarian follicles in *Xenopus laevis* from South Africa: a potential issue in endocrine testing. *Aquat Toxicol* 95:10–16
 358. Murphy MB, Hecker M, Coady KK, Tompsett AR, Jones PD, Du Preez LH, Everson GJ, Solomon KR, Carr JA, Smith EE, Kendall RJ, Van Der Kraak G, Giesy JP 2006 Atrazine concentrations, gonadal gross morphology and histology in ranid frogs collected in Michigan agricultural areas. *Aquat Toxicol* 76:230–245
 359. Suzawa M, Ingraham HA 2008 The herbicide atrazine activates endocrine gene networks via non-steroidal NR5A nuclear receptors in fish and mammalian cells. *PLoS ONE* 3:e2117
 360. Forson D, Storfer A 2006 Effects of atrazine and iridovirus infection on survival and life-history traits of the long-toed salamander (*Ambystoma macrodactylum*). *Environ Toxicol Chem* 25:168–173
 361. Forson DD, Storfer A 2006 Atrazine increases ranavirus susceptibility in the tiger salamander, *Ambystoma tigrinum*. *Ecol Appl* 16:2325–2332
 362. Rohr JR, Palmer BD 2005 Aquatic herbicide exposure increases salamander desiccation risk eight months later in a terrestrial environment. *Environ Toxicol Chem* 24:1253–1258
 363. Storrs SI, Kiesecker JM 2004 Survivorship patterns of larval amphibians exposed to low concentrations of atrazine. *Environ Health Perspect* 112:1054–1057
 364. Nieves-Puigdoller K, Björnsson BT, McCormick SD 2007 Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. *Aquat Toxicol* 84:27–37
 365. Barr DB, Panuwet P, Nguyen JV, Udunka S, Needham LL 2007 Assessing exposure to atrazine and its metabolites using biomonitoring. *Environ Health Perspect* 115:1474–1478
 366. Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, Kromhout H, Reynolds SJ, Alavanja MC 2007 Pesticide

- dose estimates for children of Iowa farmers and non-farmers. *Environ Res* 105:307–315
367. Rayner JL, Enoch RR, Fenton SE 2005 Adverse effects of prenatal exposure to atrazine during a critical period of mammary gland growth. *Toxicol Sci* 87:255–266
368. Rayner JL, Wood C, Fenton SE 2004 Exposure parameters necessary for delayed puberty and mammary gland development in Long-Evans rats exposed *in utero* to atrazine. *Toxicol Appl Pharmacol* 195:23–34
369. Cooper RL, Stoker TE, Goldman JM, Parrish MB, Tyrey L 1996 Effect of atrazine on ovarian function in the rat. *Reprod Toxicol* 10:257–264
370. Friedmann AS 2002 Atrazine inhibition of testosterone production in rat males following peripubertal exposure. *Reprod Toxicol* 16:275–279
371. Rayner JL, Enoch RR, Wolf DC, Fenton SE 2007 Atrazine-induced reproductive tract alterations after transplacental and/or lactational exposure in male Long-Evans rats. *Toxicol Appl Pharmacol* 218:238–248
372. Karrow NA, McCay JA, Brown RD, Musgrove DL, Guo TL, Germolec DR, White Jr KL 2005 Oral exposure to atrazine modulates cell-mediated immune function and decreases host resistance to the B16F10 tumor model in female B6C3F1 mice. *Toxicology* 209:15–28
373. Enoch RR, Stanko JP, Greiner SN, Youngblood GL, Rayner JL, Fenton SE 2007 Mammary gland development as a sensitive end point after acute prenatal exposure to an atrazine metabolite mixture in female Long-Evans rats. *Environ Health Perspect* 115:541–547
374. Stanko JP, Enoch RR, Rayner JL, Davis CC, Wolf DC, Malarkey DE, Fenton SE 2010 Effects of prenatal exposure to a low dose atrazine metabolite mixture on pubertal timing and prostate development of male Long-Evans rats. *Reprod Toxicol* 30:540–549
375. Schecter A, Birnbaum L, Ryan JJ, Constable JD 2006 Dioxins: an overview. *Environ Res* 101:419–428
376. Mukerjee D 1998 Health impact of polychlorinated dibenzo-*p*-dioxins: a critical review. *J Air Waste Manag Assoc* 48:157–165
377. Emond C, Birnbaum LS, DeVito MJ 2006 Use of a physiologically based pharmacokinetic model for rats to study the influence of body fat mass and induction of CYP1A2 on the pharmacokinetics of TCDD. *Environ Health Perspect* 114:1394–1400
378. Milbrath MO, Wenger Y, Chang CW, Emond C, Garabrant D, Gillespie BW, Jolliet O 2009 Apparent half-lives of dioxins, furans, and polychlorinated biphenyls as a function of age, body fat, smoking status, and breast-feeding. *Environ Health Perspect* 117:417–425
379. Emond C, Michalek JE, Birnbaum LS, DeVito MJ 2005 Comparison of the use of a physiologically based pharmacokinetic model and a classical pharmacokinetic model for dioxin exposure assessments. *Environ Health Perspect* 113:1666–1668
380. Gierthy JF, Crane D 1984 Reversible inhibition of *in vitro* epithelial cell proliferation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Appl Pharmacol* 74:91–98
381. Korkalainen M, Tuomisto J, Pohjanvirta R 2001 The AH receptor of the most dioxin-sensitive species, guinea pig, is highly homologous to the human AH receptor. *Biochem Biophys Res Commun* 285:1121–1129
382. Okey AB, Riddick DS, Harper PA 1994 The Ah receptor: mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. *Toxicol Lett* 70:1–22
383. Matsumura F 2009 The significance of the nongenomic pathway in mediating inflammatory signaling of the dioxin-activated Ah receptor to cause toxic effects. *Biochem Pharmacol* 77:608–626
384. Birnbaum LS, Tuomisto J 2000 Non-carcinogenic effects of TCDD in animals. *Food Addit Contam* 17:275–288
385. DeVito MJ, Birnbaum LS, Farland WH, Gasiewicz TA 1995 Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. *Environ Health Perspect* 103:820–831
386. Kung T, Murphy KA, White LA 2009 The aryl hydrocarbon receptor (AhR) pathway as a regulatory pathway for cell adhesion and matrix metabolism. *Biochem Pharmacol* 77:536–546
387. Li H, Wang H 2010 Activation of xenobiotic receptors: driving into the nucleus. *Expert Opin Drug Metab Toxicol* 6:409–426
388. Marinkovič N, Pašalič D, Ferencik G, Grškovič B, Stavljenič Rukavina A 2010 Dioxins and human toxicity. *Arh Hig Rada Toksikol* 61:445–453
389. White SS, Birnbaum LS 2009 An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 27:197–211
390. Swedenborg E, Pongratz I 2010 AhR and ARNT modulate ER signaling. *Toxicology* 268:132–138
391. Schwetz BA, Norris JM, Sparschu GL, Rowe UK, Gehring PJ, Emerson JL, Gerbig CG 1973 Toxicology of chlorinated dibenzo-*p*-dioxins. *Environ Health Perspect* 5:87–99
392. Kociba RJ, Schwetz BA 1982 Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Drug Metab Rev* 13:387–406
393. Couture LA, Abbott BD, Birnbaum LS 1990 A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: recent advances toward understanding the mechanism. *Teratology* 42:619–627
394. Mocarelli P, Needham LL, Marocchi A, Patterson DG Jr, Brambilla P, Gerthoux PM, Meazza L, Carreri V 1991 Serum concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and test results from selected residents of Seveso, Italy. *J Toxicol Environ Health* 32:357–366
395. Geusau A, Abraham K, Geissler K, Sator MO, Stingl G, Tschachler E 2001 Severe 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) intoxication: clinical and laboratory effects. *Environ Health Perspect* 109:865–869
396. Pohjanvirta R, Tuomisto J 1994 Short-term toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals: effects, mechanisms, and animal models. *Pharmacol Rev* 46:483–549
397. Chahoud I, Hartmann J, Rune GM, Neubert D 1992 Reproductive toxicity and toxicokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 3. Effects of single doses on the testis of male rats. *Arch Toxicol* 66:567–572
398. Mocarelli P, Gerthoux PM, Needham LL, Patterson Jr DG,

- Limonta G, Falbo R, Signorini S, Bertona M, Crespi C, Sarto C, Scott PK, Turner WE, Brambilla P 2011 Perinatal exposure to low doses of dioxin can permanently impair human semen quality. *Environ Health Perspect* 119:713–718
399. Mocarelli P, Gerthoux PM, Patterson Jr DG, Milani S, Limonta G, Bertona M, Signorini S, Tramacere P, Colombo L, Crespi C, Brambilla P, Sarto C, Carreri V, Sampson EJ, Turner WE, Needham LL 2008 Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. *Environ Health Perspect* 116:70–77
400. Foster WG, Maharaj-Briceño S, Cyr DG 2010 Dioxin-induced changes in epididymal sperm count and spermatogenesis. *Environ Health Perspect* 118:458–464
401. Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S 2010 Interpretation of studies on the developmental reproductive toxicology of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male offspring. *Food Chem Toxicol* 48:1439–1447
402. Bjerke DL, Peterson RE 1994 Reproductive toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male rats: different effects of *in utero* versus lactational exposure. *Toxicol Appl Pharmacol* 127:241–249
403. Faqi AS, Dalsenter PR, Merker HJ, Chahoud I 1998 Reproductive toxicity and tissue concentrations of low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male offspring rats exposed throughout pregnancy and lactation. *Toxicol Appl Pharmacol* 150:383–392
404. Gray Jr LE, Kelce WR, Monosson E, Ostby JS, Birnbaum LS 1995 Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. *Toxicol Appl Pharmacol* 131:108–118
405. Gray LE, Ostby JS, Kelce WR 1997 A dose-response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male Long Evans hooded rat offspring. *Toxicol Appl Pharmacol* 146:11–20
406. Ohsako S, Miyabara Y, Sakaue M, Ishimura R, Kakeyama M, Izumi H, Yonemoto J, Tohyama C 2002 Developmental stage-specific effects of perinatal 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure on reproductive organs of male rat offspring. *Toxicol Sci* 66:283–292
407. Simanainen U, Haavisto T, Tuomisto JT, Paranko J, Toppari J, Tuomisto J, Peterson RE, Viluksela M 2004 Pattern of male reproductive system effects after *in utero* and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure in three differentially TCDD-sensitive rat lines. *Toxicol Sci* 80:101–108
408. Sommer RJ, Ippolito DL, Peterson RE 1996 *In utero* and lactational exposure of the male Holtzman rat to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: decreased epididymal and ejaculated sperm numbers without alterations in sperm transit rate. *Toxicol Appl Pharmacol* 140:146–153
409. Mably TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A, Peterson RE 1992 *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 3. Effects on spermatogenesis and reproductive capability. *Toxicol Appl Pharmacol* 114:118–126
410. Wilker C, Johnson L, Safe S 1996 Effects of developmental exposure to indole-3-carbinol or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on reproductive potential of male rat offspring. *Toxicol Appl Pharmacol* 141:68–75
411. Jin MH, Hong CH, Lee HY, Kang HJ, Han SW 2010 Toxic effects of lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on development of male reproductive system: involvement of antioxidants, oxidants, and p53 protein. *Environ Toxicol* 25:1–8
412. Loeffler IK, Peterson RE 1999 Interactive effects of TCDD and p,p'-DDE on male reproductive tract development in *in utero* and lactationally exposed rats. *Toxicol Appl Pharmacol* 154:28–39
413. Rebourcet D, Odet F, Vérot A, Combe E, Meugnier E, Pesenti S, Leduque P, Déchaud H, Magre S, Le Magueresse-Battistoni B 2010 The effects of an *in utero* exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on male reproductive function: identification of Ccl5 as a potential marker. *Int J Androl* 33:413–424
414. Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S 2007 Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the developing male Wistar(Han) rat. I. No decrease in epididymal sperm count after a single acute dose. *Toxicol Sci* 99:214–223
415. Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S 2007 Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the developing male Wistar(Han) rat. II. Chronic dosing causes developmental delay. *Toxicol Sci* 99:224–233
416. Ohsako S, Miyabara Y, Nishimura N, Kurosawa S, Sakaue M, Ishimura R, Sato M, Takeda K, Aoki Y, Sone H, Tohyama C, Yonemoto J 2001 Maternal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) suppressed the development of reproductive organs of male rats: dose-dependent increase of mRNA levels of 5 α -reductase type 2 in contrast to decrease of androgen receptor in the pubertal ventral prostate. *Toxicol Sci* 60:132–143
417. Yonemoto J, Ichiki T, Takei T, Tohyama C 2005 Maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and the body burden in offspring of Long-Evans rats. *Environ Health Prev Med* 10:21–32
418. Arima A, Kato H, Ooshima Y, Tateishi T, Inoue A, Muneoka A, Ihara T, Kamimura S, Fukusato T, Kubota S, Sumida H, Yasuda M 2009 *In utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces a reduction in epididymal and ejaculated sperm number in rhesus monkeys. *Reprod Toxicol* 28:495–502
419. Yamano Y, Asano A, Ohta M, Hirata S, Shoda T, Ohyama K 2009 Expression of rat sperm flagellum-movement associated protein genes under 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treatment. *Biosci Biotechnol Biochem* 73:946–949
420. Korkalainen M, Tuomisto J, Pohjanvirta R 2004 Primary structure and inducibility by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) of aryl hydrocarbon receptor repressor in a TCDD-sensitive and a TCDD-resistant rat strain. *Biochem Biophys Res Commun* 315:123–131

421. Ishimaru N, Takagi A, Kohashi M, Yamada A, Arakaki R, Kanno J, Hayashi Y 2009 Neonatal exposure to low-dose 2,3,7,8-tetrachlorodibenzo-*p*-dioxin causes autoimmunity due to the disruption of T cell tolerance. *J Immunol* 182:6576–6586
422. Nohara K, Fujimaki H, Tsukumo S, Ushio H, Miyabara Y, Kijima M, Tohyama C, Yonemoto J 2000 The effects of perinatal exposure to low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on immune organs in rats. *Toxicology* 154:123–133
423. Lim J, DeWitt JC, Sanders RA, Watkins 3rd JB, Henshel DS 2007 Suppression of endogenous antioxidant enzymes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced oxidative stress in chicken liver during development. *Arch Environ Contam Toxicol* 52:590–595
424. Slezak BP, Hatch GE, DeVito MJ, Diliberto JJ, Slade R, Crissman K, Hassoun E, Birnbaum LS 2000 Oxidative stress in female B6C3F1 mice following acute and subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Toxicol Sci* 54:390–398
425. Hassoun EA, Wilt SC, DeVito MJ, Van Birgelen A, Alsharif NZ, Birnbaum LS, Stohs SJ 1998 Induction of oxidative stress in brain tissues of mice after subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Sci* 42: 23–27
426. Hermsen SA, Larsson S, Arima A, Muneoka A, Ihara T, Sumida H, Fukusato T, Kubota S, Yasuda M, Lind PM 2008 *In utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) affects bone tissue in rhesus monkeys. *Toxicology* 253:147–152
427. Keller JM, Huet-Hudson Y, Leamy LJ 2008 Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on molar development among non-resistant inbred strains of mice: a geometric morphometric analysis. *Growth Dev Aging* 71: 3–16
428. Kakeyama M, Sone H, Tohyama C 2008 Perinatal exposure of female rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induces central precocious puberty in the offspring. *J Endocrinol* 197:351–358
429. Shi Z, Valdez KE, Ting AY, Franczak A, Gum SL, Petroff BK 2007 Ovarian endocrine disruption underlies premature reproductive senescence following environmentally relevant chronic exposure to the aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Biol Reprod* 76:198–202
430. Gray LE, Wolf C, Mann P, Ostby JS 1997 *In utero* exposure to low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin alters reproductive development of female Long Evans hooded rat offspring. *Toxicol Appl Pharmacol* 146:237–244
431. Jenkins S, Rowell C, Wang J, Lamartiniere CA 2007 Prenatal TCDD exposure predisposes for mammary cancer in rats. *Reprod Toxicol* 23:391–396
432. Mitsui T, Sugiyama N, Maeda S, Tohyama C, Arita J 2006 Perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin suppresses contextual fear conditioning-accompanied activation of cyclic AMP response element-binding protein in the hippocampal CA1 region of male rats. *Neurosci Lett* 398:206–210
433. Seo BW, Powers BE, Widholm JJ, Schantz SL 2000 Radial arm maze performance in rats following gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Neurotoxicol Teratol* 22:511–519
434. Uemura H, Arisawa K, Hiyoshi M, Kitayama A, Takami H, Sawachika F, Dakeshita S, Nii K, Satoh H, Sumiyoshi Y, Morinaga K, Kodama K, Suzuki T, Nagai M, Suzuki T 2009 Prevalence of metabolic syndrome associated with body burden levels of dioxin and related compounds among Japan's general population. *Environ Health Perspect* 117:568–573
435. Hites RA 2011 Dioxins: an overview and history. *Environ Sci Technol* 45:16–20
436. De Groef B, Decallonne BR, Van der Geyten S, Darras VM, Bouillon R 2006 Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. *Eur J Endocrinol* 155:17–25
437. Blount BC, Valentin-Blasini L, Osterloh JD, Mauldin JP, Pirkle JL 2007 Perchlorate exposure of the US Population, 2001–2002. *J Expo Sci Environ Epidemiol* 17:400–407
438. Greer MA, Goodman G, Pleus RC, Greer SE 2002 Health effects assessment for environmental perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans. *Environ Health Perspect* 110: 927–937
439. Murray CW, Egan SK, Kim H, Beru N, Bolger PM 2008 US Food and Drug Administration's Total Diet Study: Dietary intake of perchlorate and iodine. *J Expo Sci Environ Epidemiol* 18:571–580
440. Huber DR, Blount BC, Mage DT, Letkiewicz FJ, Kumar A, Allen RH 2011 Estimating perchlorate exposure from food and tap water based on US biomonitoring and occurrence data. *J Expo Sci Environ Epidemiol* 21:395–407
441. Urbansky ET 2002 Perchlorate as an environmental contaminant. *Environ Sci Pollut Res Int* 9:187–192
442. Ginsberg GL, Hattis DB, Zoeller RT, Rice DC 2007 Evaluation of the U.S. EPA/OSWER preliminary remediation goal for perchlorate in groundwater: focus on exposure to nursing infants. *Environ Health Perspect* 115:361–369
443. Dasgupta PK, Dyke JV, Kirk AB, Jackson WA 2006 Perchlorate in the United States. Analysis of relative source contributions to the food chain. *Environ Sci Technol* 40: 6608–6614
444. Tan K, Anderson TA, Jones MW, Smith PN, Jackson WA 2004 Accumulation of perchlorate in aquatic and terrestrial plants at a field scale. *J Environ Qual* 33:1638–1646
445. Miller MD, Crofton KM, Rice DC, Zoeller RT 2009 Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. *Environ Health Perspect* 117: 1033–1041
446. Wolff J 1998 Perchlorate and the thyroid gland. *Pharmacol Rev* 50:89–105
447. Carrasco N 2000 Thyroid iodide transport: the Na⁺/I⁻ symporter (NIS). In: Braverman LE, Utiger RD, eds. *The thyroid: a fundamental and clinical text*. 8th ed. Philadelphia: Lippincott, Williams and Wilkins; 52–61
448. Nicola JP, Basquin C, Portulano C, Reyna-Neyra A, Paroder M, Carrasco N 2009 The Na⁺/I⁻ symporter mediates active iodide uptake in the intestine. *Am J Physiol Cell Physiol* 296:C654–C662
449. Vayre L, Sabourin JC, Caillou B, Ducreux M, Schlumberger M, Bidart JM 1999 Immunohistochemical analysis

- of Na⁺/I⁻ symporter distribution in human extra-thyroidal tissues. *Eur J Endocrinol* 141:382–386
450. 2007 The Na⁺/I symporter (NIS) mediates electroneutral active transport of the environmental pollutant perchlorate. *Proc Natl Acad Sci USA* 104:20250–20255
 451. Dohan O, De la Vieja A, Paroder V, Riedel C, Artani M, Reed M, Ginter CS, Carrasco N 2003 The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. *Endocr Rev* 24:48–77
 452. Mitchell AM, Manley SW, Morris JC, Powell KA, Bergert ER, Mortimer RH 2001 Sodium iodide symporter (NIS) gene expression in human placenta. *Placenta* 22:256–258
 453. Szinnai G, Lacroix L, Carré A, Guimiot F, Talbot M, Martinovic J, Delezoide AL, Vekemans M, Michiels S, Caillou B, Schlumberger M, Bidart JM, Polak M 2007 Sodium/iodide symporter (NIS) gene expression is the limiting step for the onset of thyroid function in the human fetus. *J Clin Endocrinol Metab* 92:70–76
 454. Blount BC, Rich DQ, Valentin-Blasini L, Lashley S, Ananth CV, Murphy E, Smulian JC, Spain BJ, Barr DB, Ledoux T, Hore P, Robson M 2009 Perinatal exposure to perchlorate, thiocyanate, and nitrate in New Jersey mothers and newborns. *Environ Sci Technol* 43:7543–7549
 455. Blount BC, Valentin-Blasini L 2006 Analysis of perchlorate, thiocyanate, nitrate and iodide in human amniotic fluid using ion chromatography and electrospray tandem mass spectrometry. *Anal Chim Acta* 567:87–93
 456. Borjan M, Marcella S, Blount B, Greenberg M, Zhang JJ, Murphy E, Valentin-Blasini L, Robson M 2011 Perchlorate exposure in lactating women in an urban community in New Jersey. *Sci Total Environ* 409:460–464
 457. Schier JG, Wolkin AF, Valentin-Blasini L, Belson MG, Kieszak SM, Rubin CS, Blount BC 2010 Perchlorate exposure from infant formula and comparisons with the perchlorate reference dose. *J Expo Sci Environ Epidemiol* 20: 281–287
 458. Pearce EN, Leung AM, Blount BC, Bazrafshan HR, He X, Pino S, Valentin-Blasini L, Braverman LE 2007 Breast milk iodine and perchlorate concentrations in lactating Boston-area women. *J Clin Endocrinol Metab* 92:1673–1677
 459. Kirk AB, Dyke JV, Martin CF, Dasgupta PK 2007 Temporal patterns in perchlorate, thiocyanate, and iodide excretion in human milk. *Environ Health Perspect* 115:182–186
 460. Zoeller RT, Rovet J 2004 Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *J Neuroendocrinol* 16:809–818
 461. Ghassabian A, Bongers-Schokking JJ, Henrichs J, Jaddoe VW, Visser TJ, Visser W, de Muinck Keizer-Schrama SM, Hooijkaas H, Steegers EA, Hofman A, Verhulst FC, van der Ende J, de Rijke YB, Tiemeier H 2011 Maternal thyroid function during pregnancy and behavioral problems in the offspring: the generation R study. *Pediatr Res* 69: 454–459
 462. Ghassabian A, Bongers-Schokking JJ, Henrichs J, Jaddoe VW, Visser TJ, Visser W, de Muinck Keizer-Schrama SM, Hooijkaas H, Steegers EA, Hofman A, Verhulst FC, van den Ende J, de Rijke YB, Tiemeier H 2011 Maternal thyroid function during pregnancy and parent-report problem behavior of the offspring up to age three years. *The Generation R Study. Pediatr Res* 69(5 Pt 1):454–459
 463. Murcia M, Rebagliato M, Iñiguez C, Lopez-Espinosa MJ, Estarlich M, Plaza B, Barona-Vilar C, Espada M, Vioque J, Ballester F 2011 Effect of iodine supplementation during pregnancy on infant neurodevelopment at 1 year of age. *Am J Epidemiol* 173:804–812
 464. Lawrence J, Lamm S, Braverman LE 2001 Low dose perchlorate (3 mg daily) and thyroid function. *Thyroid* 11:295
 465. Lawrence JE, Lamm SH, Pino S, Richman K, Braverman LE 2000 The effect of short-term low-dose perchlorate on various aspects of thyroid function. *Thyroid* 10:659–663
 466. Braverman LE, Pearce EN, He X, Pino S, Seeley M, Beck B, Magnani B, Blount BC, Firek A 2006 Effects of six months of daily low-dose perchlorate exposure on thyroid function in healthy volunteers. *J Clin Endocrinol Metab* 91:2721–2724
 467. National Research Council 2005 Health implications of perchlorate ingestion. Washington, DC: National Academies Press
 468. Eskenazi B, Warner M, Marks AR, Samuels S, Gerthoux PM, Vercellini P, Olive DL, Needham L, Patterson Jr D, Mocarelli P 2005 Serum dioxin concentrations and age at menopause. *Environ Health Perspect* 113:858–862
 469. Bleys J, Navas-Acien A, Laclaustra M, Pastor-Barriuso R, Menke A, Ordovas J, Stranges S, Guallar E 2009 Serum selenium and peripheral arterial disease: results from the national health and nutrition examination survey, 2003–2004. *Am J Epidemiol* 169:996–1003
 470. Hatch EE, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, Webster TF 2008 Body mass index and waist circumference: a cross-sectional study of NHANES data, 1999–2002. *Environ Health* 7:27
 471. Brucker-Davis F, Thayer K, Colborn T, Fenichel P 2002 Perchlorate: low dose exposure and susceptible populations. *Thyroid* 12:739; author reply 739–740
 472. Gibbs JP, Ahmad R, Crump KS, Houck DP, Leveille TS, Findley JE, Francis M 1998 Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function. *J Occup Environ Med* 40:1072–1082
 473. Lamm SH, Braverman LE, Li FX, Richman K, Pino S, Howarth G 1999 Thyroid health status of ammonium perchlorate workers: a cross-sectional occupational health study. *J Occup Environ Med* 41:248–260
 474. Braverman LE, He X, Pino S, Cross M, Magnani B, Lamm SH, Kruse MB, Engel A, Crump KS, Gibbs JP 2005 The effect of perchlorate, thiocyanate, and nitrate on thyroid function in workers exposed to perchlorate long-term. *J Clin Endocrinol Metab* 90:700–706
 475. Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L, Caldwell KL 2006 Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. *Environ Health Perspect* 114:1865–1871
 476. LaFranchi SH, Austin J 2007 How should we be treating children with congenital hypothyroidism? *J Pediatr Endocrinol Metab* 20:559–578
 477. Steinmaus C, Miller MD, Howd R 2007 Impact of smoking and thiocyanate on perchlorate and thyroid hormone associations in the 2001–2002 national health and nutrition examination survey. *Environ Health Perspect* 115: 1333–1338

478. Li Z, Li FX, Byrd D, Deyhle GM, Sesser DE, Skeels MR, Lamm SH 2000 Neonatal thyroxine level and perchlorate in drinking water. *J Occup Environ Med* 42:200–205
479. Li FX, Byrd DM, Deyhle GM, Sesser DE, Skeels MR, Karkowsky SR, Lamm SH 2000 Neonatal thyroid-stimulating hormone level and perchlorate in drinking water. *Teratology* 62:429–431
480. Lamm SH, Doemland M 1999 Has perchlorate in drinking water increased the rate of congenital hypothyroidism? *J Occup Environ Med* 41:409–411
481. Téllez Téllez R, Michaud Chacón P, Reyes Abarca C, Blount BC, Van Landingham CB, Crump KS, Gibbs JP 2005 Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. *Thyroid* 15:963–975
482. Buffler PA, Kelsh MA, Lau EC, Edinboro CH, Barnard JC, Rutherford GW, Daaboul JJ, Palmer L, Lorey FW 2006 Thyroid function and perchlorate in drinking water: an evaluation among California newborns, 1998. *Environ Health Perspect* 114:798–804
483. Kelsh MA, Buffler PA, Daaboul JJ, Rutherford GW, Lau EC, Barnard JC, Exuzides AK, Madl AK, Palmer LG, Lorey FW 2003 Primary congenital hypothyroidism, newborn thyroid function, and environmental perchlorate exposure among residents of a southern California community. *J Occup Environ Med* 45:1116–1127
484. Amitai Y, Winston G, Sack J, Wasser J, Lewis M, Blount BC, Valentin-Blasini L, Fisher N, Israeli A, Leventhal A 2007 Gestational exposure to high perchlorate concentrations in drinking water and neonatal thyroxine levels. *Thyroid* 17:843–850
485. Steinmaus C, Miller MD, Smith AH 2010 Perchlorate in drinking water during pregnancy and neonatal thyroid hormone levels in California. *J Occup Environ Med* 52:1217–1524
486. Brechner RJ, Parkhurst GD, Humble WO, Brown MB, Herman WH 2000 Ammonium perchlorate contamination of Colorado River drinking water is associated with abnormal thyroid function in newborns in Arizona. *J Occup Environ Med* 42:777–782
487. Crump C, Michaud P, Téllez R, Reyes C, Gonzalez G, Montgomery EL, Crump KS, Lobo G, Becerra C, Gibbs JP 2000 Does perchlorate in drinking water affect thyroid function in newborns or school-age children? *J Occup Environ Med* 42:603–612
488. Pearce EN, Spencer CA, Mestman JH, Lee RH, Bergoglio LM, Mereshian P, He X, Leung AM, Braverman LE 2011 The effect of environmental perchlorate on thyroid function in pregnant women from Cordoba, Argentina, and Los Angeles, California. *Endocr Pract* 17:412–417
489. Pearce EN, Lazarus JH, Smyth PP, He X, Dall’amico D, Parkes AB, Burns R, Smith DF, Maina A, Bestwick JP, Jooman M, Leung AM, Braverman LE 2010 Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant women. *J Clin Endocrinol Metab* 95:3207–3215
490. Gibbs JP, Van Landingham C 2008 Urinary perchlorate excretion does not predict thyroid function among pregnant women. *Thyroid* 18:807–808
491. Zoeller TR 2010 Environmental chemicals targeting thyroid. *Hormones* 9:28–40
492. Fenner-Crisp PA 2000 Endocrine modulators: risk characterization and assessment. *Toxicol Pathol* 28:438–440
493. Lucier GW 1997 Dose-response relationships for endocrine disruptors: what we know and what we don’t know. *Regul Toxicol Pharmacol* 26:34–35
494. Sheehan DM, Willingham E, Gaylor D, Bergeron JM, Crews D 1999 No threshold dose for estradiol-induced sex reversal of turtle embryos: how little is too much? *Environ Health Perspect* 107:155–159
495. Sheehan DM, vom Saal FS 1997 Low dose effects of hormones: a challenge for risk assessment. *Risk Policy Report* 4:31–39
496. Crews D, Bergeron JM, McLachlan JA 1995 The role of estrogen in turtle sex determination and the effect of PCBs. *Environ Health Perspect* 103(Suppl 7):73–77
497. vom Saal FS, Sheehan DM 1998 Challenging risk assessment. *Forum Appl Res Public Policy* 13:11–18
498. Bergeron JM, Crews D, McLachlan JA 1994 PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. *Environ Health Perspect* 102:780–781
499. Sonnenschein C, Olea N, Pasanen ME, Soto AM 1989 Negative controls of cell proliferation: human prostate cancer cells and androgens. *Cancer Res* 49:3474–3481
500. Geck P, Szelei J, Jimenez J, Lin TM, Sonnenschein C, Soto AM 1997 Expression of novel genes linked to the androgen-induced, proliferative shutoff in prostate cancer cells. *J Steroid Biochem Mol Biol* 63:211–218
501. Soto AM, Lin TM, Sakabe K, Olea N, Damassa DA, Sonnenschein C 1995 Variants of the human prostate LNCaP cell line as a tool to study discrete components of the androgen-mediated proliferative response. *Oncol Res* 7:545–558
502. Geck P, Maffini MV, Szelei J, Sonnenschein C, Soto AM 2000 Androgen-induced proliferative quiescence in prostate cancer: the role of AS3 as its mediator. *Proc Natl Acad Sci USA* 97:10185–10190
503. Soto AM, Sonnenschein C 1985 The role of estrogens on the proliferation of human breast tumor cells (MCF-7). *J Steroid Biochem* 23:87–94
504. Amara JF, Dannies PS 1983 17β -Estradiol has a biphasic effect on GH cell growth. *Endocrinology* 112:1141–1143
505. Soto AM, Sonnenschein C 2001 The two faces of Janus: sex steroids as mediators of both cell proliferation and cell death. *J Natl Cancer Inst* 93:1673–1675
506. Sonnenschein C, Soto AM 2008 Theories of carcinogenesis: an emerging perspective. *Semin Cancer Biol* 18:372–377
507. Harris H 2004 Tumour suppression: putting on the brakes. *Nature* 427:201
508. Yusuf I, Fruman DA 2003 Regulation of quiescence in lymphocytes. *Trends Immunol* 24:380–386
509. Ying QL, Wray J, Nichols J, Battle-Morera L, Doble B, Woodgett J, Cohen P, Smith A 2008 The ground state of embryonic stem cell self-renewal. *Nature* 453:519–523
510. Carroll JS, Meyer CA, Song J, Li W, Geistlinger TR, Eickhout J, Brodsky AS, Keeton EK, Fertuck KC, Hall GF, Wang Q, Bekiranov S, Sementchenko V, Fox EA, Silver PA, Gingeras TR, Liu XS, Brown M 2006 Genome-wide analysis of estrogen receptor binding sites. *Nat Genet* 38:1289–1297

511. **Maffini M, Denes V, Sonnenschein C, Soto A, Geck P** 2008 APRIN is a unique Pds5 paralog with features of a chromatin regulator in hormonal differentiation. *J Steroid Biochem Mol Biol* 108:32–43
512. **Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Ström A, Treuter E, Warner M, Gustafsson JA** 2007 Estrogen receptors: how do they signal and what are their targets. *Physiol Rev* 87:905–931
513. **Barkhem T, Nilsson S, Gustafsson JA** 2004 Molecular mechanisms, physiological consequences and pharmacological implications of estrogen receptor action. *Am J Pharmacogenomics* 4:19–28
514. **Shi YB** 2009 Dual functions of thyroid hormone receptors in vertebrate development: the roles of histone-modifying cofactor complexes. *Thyroid* 19:987–999
515. **Kang HY, Tsai MY, Chang C, Huang KE** 2003 Mechanisms and clinical relevance of androgens and androgen receptor actions. *Chang Gung Med J* 26:388–402
516. **Jeyakumar M, Webb P, Baxter JD, Scanlan TS, Katzenellenbogen JA** 2008 Quantification of ligand-regulated nuclear receptor corepressor and coactivator binding, key interactions determining ligand potency and efficacy for the thyroid hormone receptor. *Biochemistry* 47:7465–7476
517. **Nandi S** 1958 Endocrine control of mammary gland development and function in the C3H/He Crgl mouse. *J Natl Cancer Inst* 21:1039–1063
518. **Humphreys RC, Krajewska M, Krnacik S, Jaeger R, Weiher H, Krajewski S, Reed JC, Rosen JM** 1996 Apoptosis in the terminal end bud of the murine mammary gland: a mechanism of ductal morphogenesis. *Development* 122:4013–4022
519. **Haslam SZ** 1986 Mammary fibroblast influence on normal mouse mammary epithelial cell responses to estrogen in vitro. *Cancer Res* 46:310–316
520. **McGrath CM** 1983 Augmentation of the response of normal mammary epithelial cells to estradiol by mammary stroma. *Cancer Res* 43:1355–1360
521. **Sohoni P, Sumpter JP** 1998 Several environmental oestrogens are also anti-androgens. *J Endocrinol* 158:327–339
522. **Tilghman SL, Nierth-Simpson EN, Wallace R, Burow ME, McLachlan JA** 2010 Environmental hormones: Multiple pathways for response may lead to multiple disease outcomes. *Steroids* 75:520–523
523. **Ismail A, Nawaz Z** 2005 Nuclear hormone receptor degradation and gene transcription: an update. *IUBMB Life* 57:483–490
524. **Hoeck W, Rusconi S, Groner B** 1989 Down-regulation and phosphorylation of glucocorticoid receptors in cultured cells. Investigations with a monospecific antiserum against a bacterially expressed receptor fragment. *J Biol Chem* 264:14396–14402
525. **Lange CA, Shen T, Horwitz KB** 2000 Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. *Proc Natl Acad Sci USA* 97:1032–1037
526. **Nawaz Z, Lonard DM, Dennis AP, Smith CL, O'Malley BW** 1999 Proteasome-dependent degradation of the human estrogen receptor. *Proc Natl Acad Sci USA* 96:1858–1862
527. **Lin HK, Altuwaijri S, Lin WJ, Kan PY, Collins LL, Chang C** 2002 Proteasome activity is required for androgen receptor transcriptional activity via regulation of androgen receptor nuclear translocation and interaction with co-regulators in prostate cancer cells. *J Biol Chem* 277:36570–36576
528. **von Zastrow M, Kobilka BK** 1994 Antagonist-dependent and -independent steps in the mechanism of adrenergic receptor internalization. *J Biol Chem* 269:18448–18452
529. **Modrall JG, Nanamori M, Sadoshima J, Barnhart DC, Stanley JC, Neubig RR** 2001 ANG II type 1 receptor down-regulation does not require receptor endocytosis or G protein coupling. *Am J Physiol Cell Physiol* 281:C801–C809
530. **Kinyamu HK, Archer TK** 2003 Estrogen receptor-dependent proteasomal degradation of the glucocorticoid receptor is coupled to an increase in mdm2 protein expression. *Mol Cell Biol* 23:5867–5881
531. **Freedman NJ, Lefkowitz RJ** 1996 Desensitization of G protein-coupled receptors. *Recent Prog Horm Res* 51:319–351; discussion 352–353
532. **Lohse MJ** 1993 Molecular mechanisms of membrane receptor desensitization. *Biochim Biophys Acta* 1179:171–188
533. **Bohm SK, Grady EF, Bunnnett NW** 1997 Regulatory mechanisms that modulate signalling by G-protein-coupled receptors. *Biochem J* 322:1–18
534. **Shankaran H, Wiley HS, Resat H** 2007 Receptor down-regulation and desensitization enhance the information processing ability of signalling receptors. *BMC Syst Biol* 1:48
535. **Lesser B, Bruchovsky N** 1974 Effect of duration of the period after castration on the response of the rat ventral prostate to androgens. *Biochem J* 142:429–431
536. **Stormshak F, Leake R, Wertz N, Gorski J** 1976 Stimulatory and inhibitory effects of estrogen on uterine DNA synthesis. *Endocrinology* 99:1501–1511
537. **Bruchovsky N, Lesser B, Van Doorn E, Craven S** 1975 Hormonal effects on cell proliferation in rat prostate. *Vitam Horm* 33:61–102
538. **Coser KR, Chesnes J, Hur J, Ray S, Isselbacher KJ, Shioda T** 2003 Global analysis of ligand sensitivity of estrogen inducible and suppressible genes in MCF7/BUS breast cancer cells by DNA microarray. *Proc Natl Acad Sci USA* 100:13994–13999
539. **Hur J, Chesnes J, Coser KR, Lee RS, Geck P, Isselbacher KJ, Shioda T** 2004 The Bik BH3-only protein is induced in estrogen-starved and antiestrogen-exposed breast cancer cells and provokes apoptosis. *Proc Natl Acad Sci USA* 101:2351–2356
540. **Li L, Andersen ME, Heber S, Zhang Q** 2007 Non-monotonic dose-response relationship in steroid hormone receptor-mediated gene expression. *J Mol Endocrinol* 38:569–585
541. **Vandenberg LN, Wadia PR, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM** 2006 The mammary gland response to estradiol: monotonic at the cellular level, non-monotonic at the tissue-level of organization? *J Steroid Biochem Mol Biol* 101:263–274
542. **Schell LM, Burnitz KK, Lathrop PW** 2010 Pollution and human biology. *Ann Hum Biol* 37:347–366
543. **Plotkin D, Lechner JJ, Jung WE, Rosen PJ** 1978 Tamoxifen flare in advanced breast cancer. *JAMA* 240:2644–2646
544. **Osborne CK, Hobbs K, Clark GM** 1985 Effect of estrogens

- and antiestrogens on growth of human breast cancer cells in athymic nude mice. *Cancer Res* 45:584–590
545. **Berthois Y, Pons M, Dussert C, Crastes de Paulet A, Martin PM** 1994 Agonist-antagonist activity of anti-estrogens in the human breast cancer cell line MCF-7: an hypothesis for the interaction with a site distinct from the estrogen binding site. *Mol Cell Endocrinol* 99:259–268
546. **Reddel RR, Sutherland RL** 1984 Tamoxifen stimulation of human breast cancer cell proliferation in vitro: a possible model for tamoxifen tumour flare. *Eur J Cancer Clin Oncol* 20:1419–1424
547. **Wolf DM, Langan-Fahey SM, Parker CJ, McCague R, Jordan VC** 1993 Investigation of the mechanism of tamoxifen-stimulated breast tumor growth with nonisomerizable analogues of tamoxifen and metabolites. *J Natl Cancer Inst* 85:806–812
548. **Howell A** 2001 Preliminary experience with pure anti-estrogens. *Clin Cancer Res* 7:4369s–4375s; discussion 4411s–4412s
549. **Hattar R, Maller O, McDaniel S, Hansen KC, Hedman KJ, Lyons TR, Lucia S, Wilson Jr RS, Schedin P** 2009 Tamoxifen induces pleiotrophic changes in mammary stroma resulting in extracellular matrix that suppresses transformed phenotypes. *Breast Cancer Res* 11:R5
550. **Howell A, Landberg G, Bergh J** 2009 Breast tumour stroma is a prognostic indicator and target for therapy. *Breast Cancer Res* 11(Suppl 3):S16
551. **Langan-Fahey SM, Tormey DC, Jordan VC** 1990 Tamoxifen metabolites in patients on long-term adjuvant therapy for breast cancer. *Eur J Cancer* 26:883–888
552. **Kuiper GG, van den Bemd GJ, van Leeuwen JP** 1999 Estrogen receptor and the SERM concept. *J Endocrinol Invest* 22:594–603
553. **MacGregor JI, Jordan VC** 1998 Basic guide to the mechanisms of antiestrogen action. *Pharmacol Rev* 50:151–196
554. **Grese TA, Dodge JA** 1998 Selective estrogen receptor modulators (SERMs). *Curr Pharm Des* 4:71–92
555. **Nagel SC, Hagelbarger JL, McDonnell DP** 2001 Development of an ER action indicator mouse for the study of estrogens, selective ER modulators (SERMs), and xenobiotics. *Endocrinology* 142:4721–4728
556. **Gaido KW, Leonard LS, Lovell S, Gould JC, Babi D, Portier CJ, McDonnell DP** 1997 Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. *Toxicol Appl Pharmacol* 143:205–212
557. **Gould JC, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, Safe S, McDonnell DP, Gaido KW** 1998 Bisphenol A interacts with the estrogen receptor α in a distinct manner from estradiol. *Mol Cell Endocrinol* 142:203–214
558. **Lerner HJ, Band PR, Israel L, Leung BS** 1976 Phase II study of tamoxifen: report of 74 patients with stage IV breast cancer. *Cancer Treat Rep* 60:1431–1435
559. **Zhang HH, Kumar S, Barnett AH, Eggo MC** 1999 Intrinsic site-specific differences in the expression of leptin in human adipocytes and its autocrine effects on glucose uptake. *J Clin Endocrinol Metab* 84:2550–2556
560. **Haddad N, Howland R, Baroody G, Daher C** 2006 The modulatory effect of leptin on the overall insulin production in ex-vivo normal rat pancreas. *Can J Physiol Pharmacol* 84:157–162
561. **Pallett AL, Morton NM, Cawthorne MA, Emilsson V** 1997 Leptin inhibits insulin secretion and reduces insulin mRNA levels in rat isolated pancreatic islets. *Biochem Biophys Res Commun* 238:267–270
562. **Thorburn AW, Holdsworth A, Proietto J, Morahan G** 2000 Differential and genetically separable associations of leptin with obesity-related traits. *Int J Obes Relat Metab Disord* 24:742–750
563. **Lieb W, Sullivan LM, Harris TB, Roubenoff R, Benjamin EJ, Levy D, Fox CS, Wang TJ, Wilson PW, Kannel WB, Vasani RS** 2009 Plasma leptin levels and incidence of heart failure, cardiovascular disease, and total mortality in elderly individuals. *Diabetes Care* 32:612–616
564. **Neel BA, Sargis RM** 2011 The paradox of progress: environmental disruption of metabolism and the diabetes epidemic. *Diabetes* 60:1838–1848
565. **Sargis RM, Johnson DN, Choudhury RA, Brady MJ** 2010 Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. *Obesity (Silver Spring)* 18:1283–1288
566. **Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben-Jonathan N** 2008 Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environ Health Perspect* 116:1642–1647
567. **Ben-Jonathan N, Hugo ER, Brandebourg TD** 2009 Effects of bisphenol A on adipokine release from human adipose tissue: implications for the metabolic syndrome. *Mol Cell Endocrinol* 304:49–54
568. **Miyawaki J, Sakayama K, Kato H, Yamamoto H, Masuno H** 2007 Perinatal and postnatal exposure to bisphenol A increases adipose tissue mass and serum cholesterol level in mice. *J Atheroscler Thromb* 14:245–252
569. **Botelho GG, Golin M, Bufalo AC, Morais RN, Dalsenter PR, Martino-Andrade AJ** 2009 Reproductive effects of di(2-ethylhexyl)phthalate in immature male rats and its relation to cholesterol, testosterone, and thyroxine levels. *Arch Environ Contam Toxicol* 57:777–784
570. **Lutz WK, Gaylor DW, Conolly RB, Lutz RW** 2005 Non-linearity and thresholds in dose-response relationships for carcinogenicity due to sampling variation, logarithmic dose scaling, or small differences in individual susceptibility. *Toxicol Appl Pharmacol* 207:565–569
571. **Center for the Evaluation of Risks to Human Reproduction** 2007 NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Washington, DC: Department of Health and Human Services
572. **Willhite CC, Ball GL, McLellan CJ** 2008 Derivation of a Bisphenol A organ reference dose (RfD) and drinking-water equivalent concentration. *J Toxicol Environ Health B Crit Rev* 11:69–146
573. **Sakamoto H, Yokota H, Kibe R, Sayama Y, Yuasa A** 2002 Excretion of bisphenol A-glucuronide into the small intestine and deconjugation in the cecum of the rat. *Biochem Biophys Acta* 1573:171–176
574. **Zalko D, Soto AM, Dolo L, Dorio C, Rathahao E, Debrauwer L, Faure R, Cravedi JP** 2003 Biotransformations of bisphenol A in a mammalian model: answers and new

- questions raised by low-dose metabolic fate studies in pregnant CD1 mice. *Environ Health Perspect* 111:309–319
575. Stowell CL, Barvian KK, Young PC, Bigsby RM, Verdugo DE, Bertozzi CR, Widlanski TS 2006 A role for sulfation-desulfation in the uptake of bisphenol A into breast tumor cells. *Chem Biol* 13:891–897
576. Center for the Evaluation of Risks to Human Reproduction 2008 Bisphenol A: public comments. Washington, DC: Department of Health and Human Services
577. Markey CM, Michaelson CL, Veson EC, Sonnenschein C, Soto AM 2001 The mouse uterotrophic assay: a reevaluation of its validity in assessing the estrogenicity of bisphenol A. *Environ Health Perspect* 109:55–60
578. Schönfelder G, Friedrich K, Paul M, Chahoud I 2004 Developmental effects of prenatal exposure to bisphenol A on the uterus of rat offspring. *Neoplasia* 6:584–594
579. Eskenazi B, Mocarelli P, Warner M, Needham L, Patterson DG Jr, Samuels S, Turner W, Gerthoux PM, Brambilla P 2004 Relationship of serum TCDD concentrations and age at exposure of female residents of Seveso, Italy. *Environ Health Perspect* 112:22–27
580. Warner M, Eskenazi B, Mocarelli P, Gerthoux PM, Samuels S, Needham L, Patterson D, Brambilla P 2002 Serum dioxin concentrations and breast cancer risk in the Seveso Women's Health Study. *Environ Health Perspect* 110:625–628
581. Eskenazi B, Mocarelli P, Warner M, Samuels S, Vercellini P, Olive D, Needham LL, Patterson Jr DG, Brambilla P, Gavoni N, Casalini S, Panazza S, Turner W, Gerthoux PM 2002 Serum dioxin concentrations and endometriosis: a cohort study in Seveso, Italy. *Environ Health Perspect* 110:629–634
582. Eskenazi B, Warner M, Mocarelli P, Samuels S, Needham LL, Patterson DG Jr, Lippman S, Vercellini P, Gerthoux PM, Brambilla P, Olive D 2002 Serum dioxin concentrations and menstrual cycle characteristics. *Am J Epidemiol* 156:383–392
583. Robinson GW, Karpf AB, Kratochwil K 1999 Regulation of mammary gland development by tissue interaction. *J Mammary Gland Biol Neoplasia* 4:9–19
584. Medina D, Sivaraman L, Hilsenbeck SG, Conneely O, Ginger M, Rosen J, Omalle BW 2001 Mechanisms of hormonal prevention of breast cancer. *Ann NY Acad Sci* 952:23–35
585. Schulz KM, Molenda-Figueira HA, Sisk CL 2009 Back to the future: the organizational-activational hypothesis adapted to puberty and adolescence. *Horm Behav* 55:597–604
586. Schulz KM, Sisk CL 2006 Pubertal hormones, the adolescent brain, and the maturation of social behaviors: lessons from the Syrian hamster. *Mol Cell Endocrinol* 254–255:120–126
587. Primus RJ, Kellogg CK 1990 Gonadal hormones during puberty organize environment-related social interaction in the male rat. *Horm Behav* 24:311–323
588. Arase S, Ishii K, Igarashi K, Aisaki K, Yoshio Y, Matushima A, Shimohigashi Y, Arima K, Kanno J, Sugimura Y 2011 Endocrine disrupter bisphenol A increases in situ estrogen production in the mouse urogenital sinus. *Biol Reprod* 84:734–742
589. Lee DH, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs Jr DR 2010 Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study. *Environ Health Perspect* 118:1235–1242
590. Lee DH, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs Jr DR 2011 Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. *PLoS ONE* 6:e15977
591. Shin JY, Choi YY, Jeon HS, Hwang JH, Kim SA, Kang JH, Chang YS, Jacobs DR Jr, Park JY, Lee DH 2010 Low-dose persistent organic pollutants increased telomere length in peripheral leukocytes of healthy Koreans. *Mutagenesis* 25:511–516
592. MacLusky NJ, Hajszan T, Leranath C 2005 The environmental estrogen bisphenol A inhibits estradiol-induced hippocampal synaptogenesis. *Environ Health Perspect* 113:675–679
593. Della Seta D, Minder I, Dessi-Fulgheri F, Farabollini F 2005 Bisphenol-A exposure during pregnancy and lactation affects maternal behavior in rats. *Brain Res Bull* 65:255–260
594. Razzoli M, Valsecchi P, Palanza P 2005 Chronic exposure to low doses bisphenol A interferes with pair-bonding and exploration in female Mongolian gerbils. *Brain Res Bull* 65:249–254
595. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A 2006 The estrogenic effect of bisphenol A disrupts pancreatic β -cell function in vivo and induces insulin resistance. *Environ Health Perspect* 114:106–112
596. Titus-Ernstoff L, Hatch EE, Hoover RN, Palmer J, Greenberg ER, Ricker W, Kaufman R, Noller K, Herbst AL, Colton T, Hartge P 2001 Long-term cancer risk in women given diethylstilbestrol (DES) during pregnancy. *Br J Cancer* 84:126–133
597. Calle EE, Mervis CA, Thun MJ, Rodriguez C, Wingo PA, Heath Jr CW 1996 Diethylstilbestrol and risk of fatal breast cancer in a prospective cohort of US women. *Am J Epidemiol* 144:645–652
598. Small CM, DeCaro JJ, Terrell ML, Dominguez C, Cameron LL, Wirth J, Marcus M 2009 Maternal exposure to a brominated flame retardant and genitourinary conditions in male offspring. *Environ Health Perspect* 117:1175–1179
599. Goldberg JM, Falcone T 1999 Effect of diethylstilbestrol on reproductive function. *Fertil Steril* 72:1–7
600. Hatch EE, Herbst AL, Hoover RN, Noller KL, Adam E, Kaufman RH, Palmer JR, Titus-Ernstoff L, Hyer M, Hartge P, Robboy SJ 2001 Incidence of squamous neoplasia of the cervix and vagina in women exposed prenatally to diethylstilbestrol (United States). *Cancer Causes Control* 12:837–845
601. Terrell ML, Berzen AK, Small CM, Cameron LL, Wirth JJ, Marcus M 2009 A cohort study of the association between secondary sex ratio and parental exposure to polybrominated biphenyl (PBB) and polychlorinated biphenyl (PCB). *Environ Health* 8:35
602. Xu X, Dailey AB, Talbott EO, Ilacqua VA, Kearney G, Asal NR 2010 Associations of serum concentrations of organochlorine pesticides with breast cancer and prostate cancer in U.S. adults. *Environ Health Perspect* 118:60–66
603. Li DK, Zhou Z, Miao M, He Y, Qing D, Wu T, Wang J,

- Weng X, Ferber J, Herrinton LJ, Zhu Q, Gao E, Yuan W 2010 Relationship between urine bisphenol-A level and declining male sexual function. *J Androl* 31:500–506
604. Lim JS, Lee DH, Jacobs Jr DR 2008 Association of brominated flame retardants with diabetes and metabolic syndrome in the U.S. population, 2003–2004. *Diabetes Care* 31:1802–1807
605. Giordano F, Abballe A, De Felip E, di Domenico A, Ferro F, Grammatico P, Ingelido AM, Marra V, Marrocco G, Vallasciani S, Figà-Talamanca I 2010 Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. *Birth Defects Res A Clin Mol Teratol* 88:241–250
606. Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J, Calafat AM 2008 Prenatal phenol and phthalate exposures and birth outcomes. *Environ Health Perspect* 116:1092–1097
607. Sunyer J, Garcia-Esteban R, Alvarez M, Guxens M, Goñi F, Basterrechea M, Vrijheid M, Guerra S, Antó JM 2010 DDE in mothers' blood during pregnancy and lower respiratory tract infections in their infants. *Epidemiology* 21:729–735
608. World Health Organization 2002 Global assessment of the state-of-the-science of endocrine disruptors. Geneva: World Health Organization
609. Tyl RW 2009 Basic exploratory research versus guideline-compliant studies used for hazard evaluation and risk assessment: bisphenol A as a case study. *Environ Health Perspect* 117:1644–1651
610. Tyl RW 2010 In honor of the Teratology Society's 50th anniversary: the role of Teratology Society members in the development and evolution of in vivo developmental toxicity test guidelines. *Birth Defects Res C Embryo Today* 90:99–102
611. Rice C, Birnbaum LS, Cogliano J, Mahaffey K, Needham L, Rogan WJ, vom Saal FS 2003 Exposure assessment for endocrine disruptors: some considerations in the design of studies. *Environ Health Perspect* 111:1683–1690
612. Soto AM, Rubin BS, Sonnenschein C 2009 Interpreting endocrine disruption from an integrative biology perspective. *Mol Cell Endocrinol* 304:3–7
613. Heindel JJ 2008 Animal models for probing the developmental basis of disease and dysfunction paradigm. *Basic Clin Pharmacol Toxicol* 102:76–81
614. Heindel JJ, vom Saal FS 2009 Role of nutrition and environmental endocrine disrupting chemicals during the perinatal period on the aetiology of obesity. *Mol Cell Endocrinol* 304:90–96
615. Newbold RR, Padilla-Banks E, Jefferson WN, Heindel JJ 2008 Effects of endocrine disruptors on obesity. *Int J Androl* 31:201–208
616. Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J, Vickers C 2008 IPCS framework for analyzing the relevance of a noncancer mode of action for humans. *Crit Rev Toxicol* 38:87–96
617. German Federal Institute for Risk Assessment (BfR) 2009 Establishment of assessment and decision criteria in human health risk assessment for substances with endocrine disrupting properties under the EU plan protection product regulation. Report of a workshop hosted at the German Federal Institute for Risk Assessment (BfR), Berlin, Germany, 2009
618. Lidsky TI, Schneider JS 2006 Adverse effects of childhood lead poisoning: the clinical neuropsychological perspective. *Environ Res* 100:284–293
619. Sheehan DM 2006 No-threshold dose-response curves for nongenotoxic chemicals: findings and application for risk assessment. *Environ Res* 100:93–99
620. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC 2009 Endocrine-disrupting chemical: an Endocrine Society scientific statement. *Endocr Rev* 30:293–342
621. American Society of Human Genetics; American Society for Reproductive Medicine; Endocrine Society; Genetics Society of America; Society for Developmental Biology; Society for Pediatric Urology; Society for the Study of Reproduction; Society for Gynecologic Investigation 2011 Assessing chemical risk: societies offer expertise. *Science* 331:1136
622. Tominaga T, Negishi T, Hirooka H, Miyachi A, Inoue A, Hayasaka I, Yoshikawa Y 2006 Toxicokinetics of bisphenol A in rats, monkeys and chimpanzees by the LC-MS/MS method. *Toxicology* 226:208–217
623. Newbold RR 2004 Lessons learned from perinatal exposure to diethylstilbestrol. *Toxicol Appl Pharmacol* 199:142–150
624. Taylor JA, Vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, Toutain PL, Laffont CM, Vandervoort CA 2011 Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. *Environ Health Perspect* 119:422–430
625. Gies A, Heinzow B, Dieter HH, Heindel J 2009 Bisphenol A workshop of the German Federal Government Agency: March 30–31, 2009. Work group report: public health issues of bisphenol A. *Int J Hyg Environ Health* 212:693–696
626. World Health Organization 2010 Joint FAO/WHO expert meeting to review toxicological and health aspects of bisphenol A. Geneva: World Health Organization
627. Kortenkamp A 2008 Low dose mixture effects of endocrine disruptors: implications for risk assessment and epidemiology. *Int J Androl* 31:233–240
628. Bergeron JM, Willingham E, Osborn CT 3rd, Rhen T, Crews D 1999 Developmental synergism of steroidal estrogens in sex determination. *Environ Health Perspect* 107:93–97
629. Rajapakse N, Silva E, Kortenkamp A 2002 Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone activity. *Environ Health Perspect* 110:917–921
630. Rajapakse N, Silva E, Scholze M, Kortenkamp A 2004 Deviation from additivity with estrogenic mixtures containing 4-nonylphenol and 4-tert-octylphenol detected in the E-SCREEN assay. *Environ Sci Technol* 38:6343–6352
631. Kortenkamp A, Faust M, Scholze M, Backhaus T 2007 Low-level exposure to multiple chemicals: reason for human health concerns? *Environ Health Perspect* 115(Suppl 1):106–114
632. Silins I, Högberg J 2011 Combined toxic exposures and human health: biomarkers of exposure and effect. *Int J Environ Res Public Health* 8:629–647
633. Rudel RA, Gray JM, Engel CL, Rawsthorne TW, Dodson RE, Ackerman JM, Rizzo J, Nudelman JL, Brody JG 2011

- Food packaging and bisphenol A and bis(2-ethylhexyl) phthalate exposure: findings from a dietary intervention. *Environ Health Perspect* 119:914–920
634. Ji K, Kho YL, Park Y, Choi K 2010 Influence of a five-day vegetarian diet on urinary levels of antibiotics and phthalate metabolites: a pilot study with “Temple Stay” participants. *Environ Res* 110:375–382
 635. Carwile JL, Luu HT, Bassett LS, Driscoll DA, Yuan C, Chang JY, Ye X, Calafat AM, Michels KB 2009 Polycarbonate bottle use and urinary bisphenol A concentrations. *Environ Health Perspect* 117:1368–1372
 636. Matsumoto A, Kunugita N, Kitagawa K, Isse T, Oyama T, Foureman GL, Morita M, Kawamoto T 2003 Bisphenol A levels in human urine. *Environ Health Perspect* 111:101–104
 637. Kawagoshi Y, Fujita Y, Kishi I, Fukunaga I 2003 Estrogenic chemicals and estrogenic activity in leachate from municipal waste landfill determined by yeast two-hybrid assay. *J Environ Monit* 5:269–274
 638. Liao C, Kannan K 2011 High levels of bisphenol a in paper currencies from several countries, and implications for dermal exposure. *Environ Sci Technol* 45:6761–6768
 639. Lopez-Espinosa MJ, Granada A, Araque P, Molina-Molina JM, Puertollano MC, Rivas A, Fernández M, Cerrillo I, Olea-Serrano MF, López C, Olea N 2007 Oestrogenicity of paper and cardboard extracts used as food containers. *Food Addit Contam* 24:95–102
 640. Terasaki M, Shiraiishi F, Fukazawa H, Makino M 2007 Occurrence and estrogenicity of phenolics in paper-recycling process water: pollutants originating from thermal paper in waste paper. *Environ Toxicol Chem* 26:2356–2366
 641. Carson R 1962 *Silent spring*. Boston, MA: Houghton Mifflin
 642. Chung E, Genco MC, Megrelis L, Ruderman JV 2011 Effects of bisphenol A and triclocarban on brain-specific expression of aromatase in early zebrafish embryos. *Proc Natl Acad Sci USA* 108:17732–17737
 643. Rhee JS, Kim BM, Lee CJ, Yoon YD, Lee YM, Lee JS 2011 Bisphenol A modulates expression of sex differentiation genes in the self-fertilizing fish, *Kryptolebias marmoratus*. *Aquat Toxicol* 104:218–229
 644. Hatf A, Alavi SM, Abdulfatah A, Fontaine P, Rodina M, Linhart O 2012 Adverse effects of bisphenol A on reproductive physiology in male goldfish at environmentally relevant concentrations. *Ecotoxicol Environ Saf* 76:56–62
 645. Bai Y, Zhang YH, Zhai LL, Li XY, Yang J, Hong YY 2011 Estrogen receptor expression and vitellogenin synthesis induced in hepatocytes of male frogs *Rana chensinensis* exposed to bisphenol A. *Zool Res* 32:317–322
 646. Levy G, Lutz I, Krüger A, Kloas W 2004 Bisphenol A induces feminization in *Xenopus laevis* tadpoles. *Environ Res* 94:102–111
 647. Stoker C, Rey F, Rodriguez H, Ramos JG, Sirosky P, Larrera A, Luque EH, Muñoz-de-Toro M 2003 Sex reversal effects on *Caiman latirostris* exposed to environmentally relevant doses of the xenoestrogen bisphenol A. *Gen Comp Endocrinol* 133:287–296
 648. Stoker C, Beldoménico PM, Bosquiazzo VL, Zayas MA, Rey F, Rodríguez H, Muñoz-de-Toro M, Luque EH 2008 Developmental exposure to endocrine disruptor chemicals alters follicular dynamics and steroid levels in *Caiman latirostris*. *Gen Comp Endocrinol* 156:603–612
 649. Crain DA, Guillette Jr LJ, Rooney AA, Pickford DB 1997 Alterations in steroidogenesis in alligators (*Alligator mississippiensis*) exposed naturally and experimentally to environmental contaminants. *Environ Health Perspect* 105:528–533
 650. Mukhi S, Patiño R 2007 Effects of prolonged exposure to perchlorate on thyroid and reproductive function in zebrafish. *Toxicol Sci* 96:246–254
 651. Mukhi S, Torres L, Patiño R 2007 Effects of larval-juvenile treatment with perchlorate and co-treatment with thyroxine on zebrafish sex ratios. *Gen Comp Endocrinol* 150:486–494
 652. Bernhardt RR, von Hippel FA, O’Hara TM 2011 Chronic perchlorate exposure causes morphological abnormalities in developing stickleback. *Environ Toxicol Chem* 30:1468–1478
 653. Li W, Zha J, Yang L, Li Z, Wang Z 2011 Regulation of iodothyronine deiodinases and sodium iodide symporter mRNA expression by perchlorate in larvae and adult Chinese rare minnow (*Gobiocypris rarus*). *Marine Pollut Bull* 63:350–355
 654. Goleman WL, Urquidi LJ, Anderson TA, Smith EE, Kendall RJ, Carr JA 2002 Environmentally relevant concentrations of ammonium perchlorate inhibit development and metamorphosis in *Xenopus laevis*. *Environ Toxicol Chem* 21:424–430
 655. Ortiz-Santaliestra ME, Sparling DW 2007 Alteration of larval development and metamorphosis by nitrate and perchlorate in southern leopard frogs (*Rana sphenoccephala*). *Arch Environ Contam Toxicol* 53:639–646
 656. Hornung MW, Degitz SJ, Korte LM, Olson JM, Kosian PA, Linnum AL, Tietge JE 2010 Inhibition of thyroid hormone release from cultured amphibian thyroid glands by methimazole, 6-propylthiouracil, and perchlorate. *Toxicol Sci* 118:42–51
 657. Opitz R, Kloas W 2010 Developmental regulation of gene expression in the thyroid gland of *Xenopus laevis* tadpoles. *Gen Comp Endocrinol* 168:199–208
 658. Tietge JE, Butterworth BC, Haselman JT, Holcombe GW, Hornung MW, Korte JJ, Kosian PA, Wolfe M, Degitz SJ 2010 Early temporal effects of three thyroid hormone synthesis inhibitors in *Xenopus laevis*. *Aquat Toxicol* 98:44–50
 659. Chen Y, Sible JC, McNabb FMA 2008 Effects of maternal exposure to ammonium perchlorate on thyroid function and the expression of thyroid-responsive genes in Japanese quail embryos. *Gen Comp Endocrinol* 159:196–207
 660. Chen Y, McNabb FM, Sible JC 2009 Perchlorate exposure induces hypothyroidism and affects thyroid-responsive genes in liver but not brain of quail chicks. *Arch Environ Contam Toxicol* 57:598–607
 661. Pflugfelder O 1959 The alteration of the thyroid and other organs of the domestic fowl by potassium perchlorate, with comparative studies on lower vertebrates. *Wilhelm Roux Arch Entwicklungsmech Organ* 151:78–112
 662. Dent JN, Lynn WG 1958 A comparison of the effects of goitrogens on thyroid activity in *Triturus viridescens* and *Desmognathus fuscus*. *Biol Bull* 115:411–420
 663. Fox GA 2001 Wildlife as sentinels of human health effects

- in the Great Lakes–St. Lawrence basin. *Environ Health Perspect* 109(Suppl 6):853–861
664. Tanabe S 2002 Contamination and toxic effects of persistent endocrine disrupters in marine mammals and birds. *Mar Pollut Bull* 45:69–77
665. Carney SA, Prasch AL, Heideman W, Peterson RE 2006 Understanding dioxin developmental toxicity using the zebrafish model. *Birth Defects Res A Clin Mol Teratol* 76:7–18
666. Fisk AT, de Wit CA, Wayland M, Kuzyk ZZ, Burgess N, Letcher R, Braune B, Norstrom R, Blum SP, Sandau C, Lie E, Larsen HJ, Skaare JU, Muir DC 2005 An assessment of the toxicological significance of anthropogenic contaminants in Canadian arctic wildlife. *Sci Total Environ* 351–352:57–93
667. Cooper KR, Wintermyer M 2009 A critical review: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) effects on gonad development in bivalve mollusks. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 27:226–245
668. Van den Berg M, Birnbaum L, Bosveld AT, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FX, Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T 1998 Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106:775–792
669. Gray LE, Ostby J, Wolf C, Lambright C, Kelce W 1998 The value of mechanistic studies in laboratory animals for the prediction of reproductive effects in wildlife: endocrine effects on mammalian sexual differentiation. *Environ Toxicol Chem* 17:109–118
670. Hayes TB 1998 Endocrine disruptors in amphibians: potential impacts and the usefulness of amphibian screens for detecting endocrine disrupting compounds. *Sci J (Kagaku)* 68:557–568
671. Colborn T 1994 The wildlife/human connection: modernizing risk decisions. *Environ Health Perspect* 102:55–59
672. Colborn T 1995 Environmental estrogens: health implications for humans and wildlife. *Environ Health Perspect* 103:135–136
673. Harrison PT, Holmes P, Humfrey CD 1997 Reproductive health in humans and wildlife: are adverse trends associated with environmental chemical exposure? *Sci Total Environ* 205:97–106
674. Edwards TM, Moore BC, Guillette Jr LJ 2006 Reproductive dysgenesis in wildlife: a comparative view. *Int J Androl* 29:109–121
675. Rhind SM 2009 Anthropogenic pollutants: a threat to ecosystem sustainability? *Philos Trans R Soc Lond B Biol Sci* 364:3391–3401
676. Decensi A, Gandini S, Guerrieri-Gonzaga A, Johansson H, Manetti L, Bonanni B, Sandri MT, Barreca A, Costa A, Robertson C, Lien EA 1999 Effect of blood tamoxifen concentrations on surrogate biomarkers in a trial of dose reduction in healthy women. *J Clin Oncol* 17:2633–2638
677. Kisanga ER, Gjerde J, Guerrieri-Gonzaga A, Pigatto F, Pesci-Feltri A, Robertson C, Serrano D, Pelosi G, Decensi A, Lien EA 2004 Tamoxifen and metabolite concentrations in serum and breast cancer tissue during three dose regimens in a randomized preoperative trial. *Clin Cancer Res* 10:2336–2343
678. Nagel SC, vom Saal FS, Welshons WV 1998 The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: physiology of delivery modifies estrogenic activity. *Proc Soc Exp Biol Med* 217:300–309
679. Lakind JS, Naiman DQ 2008 Bisphenol A (BPA) daily intakes in the United States: estimates from the 2003–2004 NHANES urinary BPA data. *J Expo Sci Environ Epidemiol* 18:608–615
680. Wittassek M, Koch HM, Angerer J, Brüning T 2011 Assessing exposure to phthalates: the human biomonitoring approach. *Mol Nutr Food Res* 55:7–31
681. David RM, Moore MR, Finney DC, Guest D 2000 Chronic toxicity of di(2-ethylhexyl)phthalate in rats. *Toxicol Sci* 55:433–443
682. Agency for Toxic Substances and Diseases Registry 2011 Toxic substances portal: di(2-ethylhexyl)phthalate (DEHP). Atlanta, GA: Centers for Disease Control
683. Dickerson SM, Cunningham SL, Patisaul HB, Woller MJ, Gore AC 2011 Endocrine disruption of brain sexual differentiation by developmental PCB exposure. *Endocrinology* 152:581–594
684. Salama J, Chakraborty TR, Ng L, Gore AC 2003 Effects of polychlorinated biphenyls on estrogen receptor- β expression in the anteroventral periventricular nucleus. *Environ Health Perspect* 111:1278–1282
685. Cassidy RA, Vorhees CV, Minnema DJ, Hastings L 1994 The effects of chlordane exposure during pre- and postnatal periods at environmentally relevant levels on sex steroid-mediated behaviors and functions in the rat. *Toxicol Appl Pharmacol* 126:326–337
686. McMahon T, Halstead N, Johnson S, Raffel TR, Romanic JM, Crumrine PW, Boughton RK, Martin LB, Rohr JR 2011 The fungicide chlorothalonil is nonlinearly associated with corticosterone levels, immunity, and mortality in amphibians. *Environ Health Perspect* 119:1098–1103
687. Guo-Ross SX, Chambers JE, Meek EC, Carr RL 2007 Altered muscarinic acetylcholine receptor subtype binding in neonatal rat brain following exposure to chlorpyrifos or methyl parathion. *Toxicol Sci* 100:118–127
688. Palanza P, Parmigiani S, Liu H, vom Saal FS 1999 Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and *o,p'*-DDT alters aggressive behavior of male and female house mice. *Pharmacol Biochem Behav* 64:665–672
689. vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S, Welshons WV 1997 Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. *Proc Natl Acad Sci USA* 94:2056–2061
690. Slikker Jr W, Scallet AC, Doerge DR, Ferguson SA 2001 Gender-based differences in rats after chronic dietary exposure to genistein. *Int J Toxicol* 20:175–179
691. Smialowicz RJ, Williams WC, Copeland CB, Harris MW, Overstreet D, Davis BJ, Chapin RE 2001 The effects of perinatal/juvenile heptachlor exposure on adult immune and reproductive system function in rats. *Toxicol Sci* 61:164–175

692. Valkusz Z, Nagyéri G, Radács M, Ocskó T, Hausinger P, László M, László FA, Juhász A, Julesz J, Pálföldi R, Gálfi M 2011 Further analysis of behavioral and endocrine consequences of chronic exposure of male Wistar rats to subtoxic doses of endocrine disruptor chlorobenzenes. *Physiol Behav* 103:421–430
693. Manfo FP, Chao WF, Moundipa PF, Pugeat M, Wang PS 2011 Effects of maneb on testosterone release in male rats. *Drug Chem Toxicol* 34:120–128
694. Chapin RE, Harris MW, Davis BJ, Ward SM, Wilson RE, Mauney MA, Lockhart AC, Smialowicz RJ, Moser VC, Burka LT, Collins BJ 1997 The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. *Fundam Appl Toxicol* 40:138–157
695. White Jr KL, Germolec DR, Booker CD, Hernandez DM, McCay JA, Delclos KB, Newbold RR, Weis C, Guo TL 2005 Dietary methoxychlor exposure modulates splenic natural killer cell activity, antibody-forming cell response and phenotypic marker expression in F0 and F1 generations of Sprague Dawley rats. *Toxicology* 207:271–281
696. Faass O, Schlumpf M, Reolon S, Henseler M, Maerkel K, Durrer S, Lichtensteiger W 2009 Female sexual behavior, estrous cycle and gene expression in sexually dimorphic brain regions after pre- and postnatal exposure to endocrine active UV filters. *Neurotoxicology* 30:249–260
697. Lemini C, Hernández A, Jaimez R, Franco Y, Avila ME, Castell A 2004 Morphometric analysis of mice uteri treated with the preservatives methyl, ethyl, propyl, and butylparaben. *Toxicol Ind Health* 20:123–132
698. Damgaard IN, Jensen TK, Petersen JH, Skakkebaek NE, Toppari J, Main KM 2008 Risk factors for congenital cryptorchidism in a prospective birth cohort study. *PLoS ONE* 3:e3051
699. Laurenzana EM, Weis CC, Bryant CW, Newbold R, Delclos KB 2002 Effect of dietary administration of genistein, nonylphenol or ethinyl estradiol on hepatic testosterone metabolism, cytochrome P-450 enzymes, and estrogen receptor α expression. *Food Chem Toxicol* 40:53–63
700. Tyl RW, Myers CB, Marr MC, Brine DR, Fail PA, Seely JC, Van Miller JP 1999 Two-generation reproduction study with para-tert-octylphenol in rats. *Regul Toxicol Pharmacol* 30:81–95
701. Li E, Guo Y, Ning Q, Zhang S, Li D 2011 Research for the effect of octylphenol on spermatogenesis and proteomic analysis in octylphenol-treated mice testes. *Cell Biol Int* 35:305–309
702. Timofeeva OA, Sanders D, Seemann K, Yang L, Hermanson D, Regenbogen S, Agoos S, Kallepalli A, Rastogi A, Braddy D, Wells C, Perraut C, Scidler FJ, Slotkin TA, Levin ED 2008 Persistent behavioral alterations in rats neonatally exposed to low doses of the organophosphate pesticide, parathion. *Brain Res Bull* 77:404–411
703. Kuriyama SN, Wanner A, Fidalgo-Neto AA, Talsness CE, Koerner W, Chahoud I 2007 Developmental exposure to low-dose PBDE-99: tissue distribution and thyroid hormone levels. *Toxicology* 242:80–90
704. Tanaka T, Morita A, Kato M, Hirai T, Mizoue T, Terauchi Y, Watanabe S, Noda M 2011 Congener-specific polychlorinated biphenyls and the prevalence of diabetes in the Saku Control Obesity Program (SCOP). *Endocr J* 58:589–596
705. Buckman AH, Fisk AT, Parrott JL, Solomon KR, Brown SB 2007 PCBs can diminish the influence of temperature on thyroid indices in rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 84:366–378
706. Jiang Y, Zhao J, Van Audekercke R, Dequeker J, Geusens P 1996 Effects of low-dose long-term sodium fluoride preventive treatment on rat bone mass and biomechanical properties. *Calcif Tissue Int* 58:30–39
707. Kirchner S, Kieu T, Chow C, Casey S, Blumberg B 2010 Prenatal exposure to the environmental obesogen tributyltin predisposes multipotent stem cells to become adipocytes. *Mol Endocrinol* 24:526–539
708. Stoker TE, Gibson EK, Zorrilla LM 2010 Triclosan exposure modulates estrogen-dependent responses in the female wistar rat. *Toxicol Sci* 117:45–53
709. Eustache F, Mondon F, Canivenc-Lavier MC, Lesaffre C, Fulla Y, Berges R, Cravedi JP, Vaiman D, Auger J 2009 Chronic dietary exposure to a low-dose mixture of genistein and vinclozolin modifies the reproductive axis, testis transcriptome, and fertility. *Environ Health Perspect* 117:1272–1279
710. Schlumpf M, Durrer S, Faass O, Ehnes C, Fuetsch M, Gaille C, Henseler M, Hofkamp L, Maerkel K, Reolon S, Timms B, Tresguerres JA, Lichtensteiger W 2008 Developmental toxicity of UV filters and environmental exposure: a review. *Int J Androl* 31:144–151
711. Schlecht C, Klammer H, Wuttke W, Jarry H 2006 A dose-response study on the estrogenic activity of benzophenone-2 on various endpoints in the serum, pituitary and uterus of female rats. *Arch Toxicol* 80:656–661
712. Sitarek K 2001 Embryo-lethal and teratogenic effects of carbendazim in rats. *Teratog Carcinog Mutagen* 21:335–340
713. Higashihara N, Shiraishi K, Miyata K, Oshima Y, Minobe Y, Yamasaki K 2007 Subacute oral toxicity study of bisphenol F based on the draft protocol for the “Enhanced OECD Test Guideline no. 407”. *Arch Toxicol* 81:825–832
714. Yamano Y, Ohyama K, Ohta M, Sano T, Ritani A, Shimada J, Ashida N, Yoshida E, Ikehara K, Morishima I 2005 A novel spermatogenesis related factor-2 (SRF-2) gene expression affected by TCDD treatment. *Endocr J* 52:75–81
715. Ikeda M, Tamura M, Yamashita J, Suzuki C, Tomita T 2005 Repeated *in utero* and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure affects male gonads in offspring, leading to sex ratio changes in F2 progeny. *Toxicol Appl Pharmacol* 206:351–355
716. Welshons WV, Nagel SC, Thayer KA, Judy BM, Vom Saal FS 1999 Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. *Toxicol Ind Health* 15:12–25
717. Christian M, Gillies G 1999 Developing hypothalamic dopaminergic neurones as potential targets for environmental estrogens. *J Endocrinol* 160:R1–R6
718. Jeng YJ, Watson CS 2011 Combinations of physiologic estrogens with xenoestrogens alter ERK phosphorylation

- profiles in rat pituitary cells. *Environ Health Perspect* 119: 104–112
719. Jeng YJ, Kochukov MY, Watson CS 2009 Membrane estrogen receptor- α -mediated nongenomic actions of phytoestrogens in GH3/B6/F10 pituitary tumor cells. *J Mol Signal* 4:2
720. Narita S, Goldblum RM, Watson CS, Brooks EG, Estes DM, Curran EM, Midoro-Horiuti T 2007 Environmental estrogens induce mast cell degranulation and enhance IgE-mediated release of allergic mediators. *Environ Health Perspect* 115:48–52
721. Somjen D, Kohen F, Jaffe A, Amir-Zaltsman Y, Knoll E, Stern N 1998 Effects of gonadal steroids and their antagonists on DNA synthesis in human vascular cells. *Hypertension* 32:39–45
722. Devidze N, Fujimori K, Urade Y, Pfaff DW, Mong JA 2010 Estradiol regulation of lipocalin-type prostaglandin D synthase promoter activity: evidence for direct and indirect mechanisms. *Neurosci Lett* 474:17–21
723. Du J, Wang Y, Hunter R, Wei Y, Blumenthal R, Falke C, Khairova R, Zhou R, Yuan P, Machado-Vieira R, McEwen BS, Manji HK 2009 Dynamic regulation of mitochondrial function by glucocorticoids. *Proc Natl Acad Sci USA* 106: 3543–3548
724. Guillen C, Bartolomé A, Nevado C, Benito M 2008 Biphasic effect of insulin on β cell apoptosis depending on glucose deprivation. *FEBS Lett* 582:3855–3860
725. Welsh Jr TH, Kasson BG, Hsueh AJ 1986 Direct biphasic modulation of gonadotropin-stimulated testicular androgen biosynthesis by prolactin. *Biol Reprod* 34:796–804
726. Sarkar PK 2008 L-Triiodothyronine differentially and non-genomically regulates synaptosomal protein phosphorylation in adult rat brain cerebral cortex: role of calcium and calmodulin. *Life Sci* 82:920–927
727. Calvo RM, Obregon MJ 2009 Tri-iodothyronine upregulates adiponitrin mRNA expression in rat and human adipocytes. *Mol Cell Endocrinol* 311:39–46
728. Leung LY, Kwong AK, Man AK, Woo NY 2008 Direct actions of cortisol, thyroxine and growth hormone on IGF-I mRNA expression in sea bream hepatocytes. *Comp Biochem Physiol A Mol Integr Physiol* 151:705–710
729. Habauzit D, Boudot A, Kerdivel G, Flouriot G, Pakdel F 2010 Development and validation of a test for environmental estrogens: checking xeno-estrogen activity by CXCL12 secretion in breast cancer cell lines (CXCL-test). *Environ Toxicol* 25:495–503
730. Boettcher M, Kosmehl T, Braunbeck T 2011 Low-dose effects and biphasic effect profiles: Is trenbolone a genotoxicant? *Mutat Res* 723:152–157
731. Wetherill YB, Petre CE, Monk KR, Puga A, Knudsen KE 2002 The xenoestrogen bisphenol A induces inappropriate androgen receptor activation and mitogenesis in prostatic adenocarcinoma cells. *Mol Cancer Ther* 1:515–524
732. Sandy EH, Yao J, Zheng S, Gogra AB, Chen H, Zheng H, Yormah TB, Zhang X, Zaray G, Ceccanti B, Choi MM 2010 A comparative cytotoxicity study of isomeric alkylphthalates to metabolically variant bacteria. *J Hazard Mater* 182:631–639
733. Murono EP, Derk RC, de León JH 1999 Biphasic effects of octylphenol on testosterone biosynthesis by cultured Leydig cells from neonatal rats. *Reprod Toxicol* 13:451–462
734. Beníšek M, Bláha L, Hilscherová K 2008 Interference of PAHs and their N-heterocyclic analogs with signaling of retinoids in vitro. *Toxicol In Vitro* 22:1909–1917
735. Beníšek M, Kubincová P, Bláha L, Hilscherová K 2011 The effects of PAHs and N-PAHs on retinoid signaling and Oct-4 expression in vitro. *Toxicol Lett* 200:169–175
736. Evanson M, Van Der Kraak GJ 2001 Stimulatory effects of selected PAHs on testosterone production in goldfish and rainbow trout and possible mechanisms of action. *Comp Biochem Physiol C Toxicol Pharmacol* 130:249–258
737. Chaube R, Mishra S, Singh RK 2010 In vitro effects of lead nitrate on steroid profiles in the post-vitellogenic ovary of the catfish *Heteropneustes fossilis*. *Toxicol In Vitro* 24: 1899–1904
738. Helmestam M, Stavreus-Evers A, Olovsson M 2010 Cadmium chloride alters mRNA levels of angiogenesis related genes in primary human endometrial endothelial cells grown in vitro. *Reprod Toxicol* 30:370–376
739. Chen AC, Donovan SM 2004 Genistein at a concentration present in soy infant formula inhibits Caco-2BBE cell proliferation by causing G2/M cell cycle arrest. *J Nutr* 134: 1303–1308
740. El Touny LH, Banerjee PP 2009 Identification of a biphasic role for genistein in the regulation of prostate cancer growth and metastasis. *Cancer Res* 69:3695–3703
741. Guo JM, Xiao BX, Liu DH, Grant M, Zhang S, Lai YF, Guo YB, Liu Q 2004 Biphasic effect of daidzein on cell growth of human colon cancer cells. *Food Chem Toxicol* 42:1641–1646
742. Wang H, Zhou H, Zou Y, Liu Q, Guo C, Gao G, Shao C, Gong Y 2010 Resveratrol modulates angiogenesis through the GSK3 β / β -catenin/TCF-dependent pathway in human endothelial cells. *Biochem Pharmacol* 80:1386–1395
743. Pedro M, Lourenço CF, Cidade H, Kijjoa A, Pinto M, Nascimento MS 2006 Effects of natural prenylated flavones in the phenotypical ER (+) MCF-7 and ER (–) MDA-MB-231 human breast cancer cells. *Toxicol Lett* 164:24–36
744. Almstrup K, Fernández MF, Petersen JH, Olea N, Skakkebaek NE, Leffers H 2002 Dual effects of phytoestrogens result in U-shaped dose-response curves. *Environ Health Perspect* 110:743–748
745. Pinto B, Bertoli A, Nocchioli C, Garritano S, Reali D, Pistelli L 2008 Estradiol-antagonistic activity of phenolic compounds from leguminous plants. *Phytother Res* 22:362–366
746. Sanderson JT, Hordijk J, Denison MS, Springsteel MF, Nantz MH, van den Berg M 2004 Induction and inhibition of aromatase (CYP19) activity by natural and synthetic flavonoid compounds in H295R human adrenocortical carcinoma cells. *Toxicol Sci* 82:70–79
747. Elattar TM, Virji AS 2000 The inhibitory effect of curcumin, genistein, quercetin and cisplatin on the growth of oral cancer cells in vitro. *Anticancer Res* 20:1733–1738
748. Ahn NS, Hu H, Park JS, Park JS, Kim JS, An S, Kong G, Aruoma OI, Lee YS, Kang KS 2005 Molecular mechanisms of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced inverted U-shaped dose responsiveness in anchorage independent growth and cell proliferation of human breast epithelial cells with stem cell characteristics. *Mutat Res* 579: 189–199
749. Dickerson SM, Guevara E, Woller MJ, Gore AC 2009 Cell

- death mechanisms in GT1–7 GnRH cells exposed to polychlorinated biphenyls PCB74, PCB118, PCB153. *Toxicol Appl Pharmacol* 237:237–245
750. Campagna C, Ayotte P, Sirard MA, Arseneault G, Laforest JP, Bailey JL 2007 Effect of an environmentally relevant metabolized organochlorine mixture on porcine cumulus-oocyte complexes. *Reprod Toxicol* 23:145–152
751. Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE 2009 Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology* 262:184–191
752. Greenman SB, Rutten MJ, Fowler WM, Scheffler L, Shortridge LA, Brown B, Sheppard BC, Deveney KE, Deveney CW, Trunkey DD 1997 Herbicide/pesticide effects on intestinal epithelial growth. *Environ Res* 75:85–93
753. Sreeramulu K, Liu R, Sharom FJ 2007 Interaction of insecticides with mammalian P-glycoprotein and their effect on its transport function. *Biochim Biophys Acta* 1768:1750–1757
754. Asp V, Ullerås E, Lindström V, Bergström U, Oskarsson A, Brandt I 2010 Biphasic hormonal responses to the adrenocorticolytic DDT metabolite 3-methylsulfonyl-DDE in human cells. *Toxicol Appl Pharmacol* 242:281–289
755. Ralph JL, Orgebin-Crist MC, Lareyre JJ, Nelson CC 2003 Disruption of androgen regulation in the prostate by the environmental contaminant hexachlorobenzene. *Environ Health Perspect* 111:461–466
756. Ohlsson A, Ullerås E, Oskarsson A 2009 A biphasic effect of the fungicide prochloraz on aldosterone, but not cortisol, secretion in human adrenal H295R cells: underlying mechanisms. *Toxicol Lett* 191:174–180
757. Ohlsson A, Cedergreen N, Oskarsson A, Ullerås E 2010 Mixture effects of imidazole fungicides on cortisol and aldosterone secretion in human adrenocortical H295R cells. *Toxicology* 275:21–28
758. Kim KH, Bose DD, Ghogha A, Riehl J, Zhang R, Barnhart CD, Lein PJ, Pessah IN 2011 Para- and ortho-substitutions are key determinants of polybrominated diphenyl ether activity toward ryanodine receptors and neurotoxicity. *Environ Health Perspect* 119:519–526
759. Alm H, Scholz B, Kultima K, Nilsson A, Andrén PE, Savitski MM, Bergman A, Stigson M, Fex-Svenningsen A, Dencker L 2010 In vitro neurotoxicity of PBDE-99: immediate and concentration-dependent effects on protein expression in cerebral cortex cells. *J Proteome Res* 9:1226–1235
760. Sánchez JJ, Abreu P, González-Hernández T, Hernández A, Prieto L, Alonso R 2004 Estrogen modulation of adrenoceptor responsiveness in the female rat pineal gland: differential expression of intracellular estrogen receptors. *J Pineal Res* 37:26–35
761. Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL 1996 Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. *Environ Health Perspect* 104:1296–1300
762. Dhir A, Kulkarni SK 2008 Antidepressant-like effect of 17 β -estradiol: involvement of dopaminergic, serotonergic, and (or) sigma-1 receptor systems. *Can J Physiol Pharmacol* 86:726–735
763. Ribeiro AC, Pfaff DW, Devidze N 2009 Estradiol modulates behavioral arousal and induces changes in gene expression profiles in brain regions involved in the control of vigilance. *Eur J Neurosci* 29:795–801
764. Park CR, Campbell AM, Woodson JC, Smith TP, Fleshner M, Diamond DM 2006 Permissive influence of stress in the expression of a U-shaped relationship between serum corticosterone levels and spatial memory errors in rats. *Dose Response* 4:55–74
765. Abrahám I, Harkany T, Horvath KM, Veenema AH, Penke B, Nyakas C, Luiten PG 2000 Chronic corticosterone administration dose-dependently modulates A β (1–42)- and NMDA-induced neurodegeneration in rat magnocellular nucleus basalis. *J Neuroendocrinol* 12:486–494
766. Duclos M, Gouarne C, Martin C, Rocher C, Mormède P, Letellier T 2004 Effects of corticosterone on muscle mitochondria identifying different sensitivity to glucocorticoids in Lewis and Fischer rats. *Am J Physiol Endocrinol Metab* 286:E159–E167
767. Abrari K, Rashidy-Pour A, Semnani S, Fathollahi Y, Javid M 2009 Post-training administration of corticosterone enhances consolidation of contextual fear memory and hippocampal long-term potentiation in rats. *Neurobiol Learn Mem* 91:260–265
768. Spée M, Marchal L, Thierry AM, Chastel O, Enstipp M, Maho YL, Beaulieu M, Raclot T 2011 Exogenous corticosterone mimics a late fasting stage in captive Adelle penguins (*Pygoscelis adeliae*). *Am J Physiol Regul Integr Comp Physiol* 300:R1241–R1249
769. Sunny F, Oommen VO 2004 Effects of steroid hormones on total brain Na⁺-K⁺ ATPase activity in *Oreochromis mossambicus*. *Indian J Exp Biol* 42:283–287
770. Huggard D, Khakoo Z, Kassam G, Mahmoud SS, Habibi HR 1996 Effect of testosterone on maturational gonadotropin subunit messenger ribonucleic acid levels in the goldfish pituitary. *Biol Reprod* 54:1184–1191
771. Ren SG, Huang Z, Sweet DE, Malozowski S, Cassorla F 1990 Biphasic response of rat tibial growth to thyroxine administration. *Acta Endocrinol (Copenh)* 122:336–340
772. Houshmand F, Faghihi M, Zahediasl S 2009 Biphasic protective effect of oxytocin on cardiac ischemia/reperfusion injury in anaesthetized rats. *Peptides* 30:2301–2308
773. Boccia MM, Kopf SR, Baratti CM 1998 Effects of a single administration of oxytocin or vasopressin and their interactions with two selective receptor antagonists on memory storage in mice. *Neurobiol Learn Mem* 69:136–146
774. Tai SH, Hung YC, Lee EJ, Lee AC, Chen TY, Shen CC, Chen HY, Lee MY, Huang SY, Wu TS 2011 Melatonin protects against transient focal cerebral ischemia in both reproductively active and estrogen-deficient female rats: the impact of circulating estrogen on its hormetic dose-response. *J Pineal Res* 50:292–303
775. Cai JX, Arnsten AF 1997 Dose-dependent effects of the dopamine D1 receptor agonists A77636 or SKF81297 on spatial working memory in aged monkeys. *J Pharmacol Exp Ther* 283:183–189
776. Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF 2007 Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat Neurosci* 10:376–384
777. Palanza P, Parmigiani S, vom Saal FS 2001 Effects of prenatal exposure to low doses of diethylstilbestrol, o,p'-DDT,

- and methoxychlor on postnatal growth and neurobehavioral development in male and female mice. *Horm Behav* 40:252–265
778. Thuillier R, Wang Y, Culty M 2003 Prenatal exposure to estrogenic compounds alters the expression pattern of platelet-derived growth factor receptors α and β in neonatal rat testis: identification of gonocytes as targets of estrogen exposure. *Biol Reprod* 68:867–880
779. Köhlerová E, Skarda J 2004 Mouse bioassay to assess oestrogenic and anti-oestrogenic compounds: hydroxytamoxifen, diethylstilbestrol and genistein. *J Vet Med A Physiol Pathol Clin Med* 51:209–217
780. Putz O, Schwartz CB, Kim S, LeBlanc GA, Cooper RL, Prins GS 2001 Neonatal low- and high-dose exposure to estradiol benzoate in the male rat. I. Effects on the prostate gland. *Biol Reprod* 65:1496–1505
781. Rochester JR, Forstmeier W, Millam JR 2010 Post-hatch oral estrogen in zebra finches (*Taeniopygia guttata*): is infertility due to disrupted testes morphology or reduced copulatory behavior? *Physiol Behav* 101:13–21
782. Vosges M, Le Page Y, Chung BC, Combarrous Y, Porcher JM, Kah O, Brion F 2010 17α -Ethinylestradiol disrupts the ontogeny of the forebrain GnRH system and the expression of brain aromatase during early development of zebrafish. *Aquat Toxicol* 99:479–491
783. Gust M, Buronfosse T, Giamberini L, Ramil M, Mons R, Garric J 2009 Effects of fluoxetine on the reproduction of two prosobranch mollusks: *Potamopyrgus antipodarum* and *Valvata piscinalis*. *Environ Pollut* 157:423–429
784. Villeneuve DL, Knoebi I, Kahl MD, Jensen KM, Hammermeister DE, Greene KJ, Blake LS, Ankley GT 2006 Relationship between brain and ovary aromatase activity and isoform-specific aromatase mRNA expression in the fathead minnow (*Pimephales promelas*). *Aquat Toxicol* 76:353–368
785. Jones BA, Shimell JJ, Watson NV 2011 Pre- and postnatal Bisphenol A treatment results in persistent deficits in the sexual behavior of male rats, but not female rats, in adulthood. *Horm Behav* 59:246–251
786. Lemos MF, Esteves AC, Samyn B, Timperman I, van Beuemen J, Correia A, van Gestel CA, Soares AM 2010 Protein differential expression induced by endocrine disrupting compounds in a terrestrial isopod. *Chemosphere* 79:570–576
787. Nishizawa H, Morita M, Sugimoto M, Imanishi S, Manabe N 2005 Effects of *in utero* exposure to bisphenol A on mRNA expression of arylhydrocarbon and retinoid receptors in murine embryos. *J Reprod Dev* 51:315–324
788. Andrade AJ, Grande SW, Talsness CE, Grote K, Chahoud I 2006 A dose-response study following *in utero* and lactational exposure to di-(2-ethylhexyl)-phthalate (DEPH): non-monotonic dose-response and low dose effects on rat brain aromatase activity. *Toxicology* 227:185–192
789. Ge RS, Chen GR, Dong Q, Akingbemi B, Sottas CM, Santos M, Sealfon SC, Bernard DJ, Hardy MP 2007 Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. *J Androl* 28:513–520
790. Grande SW, Andrade AJ, Talsness CE, Grote K, Chahoud I 2006 A dose-response study following *in utero* and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. *Toxicol Sci* 91:247–254
791. Vo TT, Jung EM, Dang VH, Yoo YM, Choi KC, Yu FH, Jeung EB 2009 Di-(2 ethylhexyl) phthalate and flutamide alter gene expression in the testis of immature male rats. *Reprod Biol Endocrinol* 7:104
792. Takano H, Yanagisawa R, Inoue K, Ichinose T, Sadakane K, Yoshikawa T 2006 Di-(2-ethylhexyl) phthalate enhances atopic dermatitis-like skin lesions in mice. *Environ Health Perspect* 114:1266–1269
793. Oliveira-Filho EC, Grisolia CK, Paumgarten FJR 2009 Trans-generation study of the effects of nonylphenol ethoxylate on the reproduction of the snail *Biomphalaria tenagophila*. *Ecotoxicol Environ Saf* 72:458–465
794. Duft M, Schulte-Oehlmann U, Weltje L, Tillmann M, Oehlmann J 2003 Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail *Potamopyrgus antipodarum*. *Aquat Toxicol* 64:437–449
795. Oehlmann J, Schulte-Oehlmann U, Tillmann M, Markert B 2000 Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I. bisphenol A and octylphenol as xeno-estrogens. *Ecotoxicology* 9:383–397
796. Maranghi F, Tassinari R, Marcoccia D, Altieri I, Catone T, De Angelis G, Testai E, Mastrangelo S, Evandri MG, Bolle P, Lorenzetti S 2010 The food contaminant semicarbazide acts as an endocrine disrupter: evidence from an integrated in vivo/in vitro approach. *Chem Biol Interact* 183:40–48
797. Giudice BD, Young TM 2010 The antimicrobial triclocarban stimulates embryo production in the freshwater mudsnail *Potamopyrgus antipodarum*. *Environ Toxicol Chem* 29:966–970
798. Love OP, Shutt LJ, Silfies JS, Bortolotti GR, Smits JE, Bird DM 2003 Effects of dietary PCB exposure on adrenocortical function in captive American kestrels (*Falco sparverius*). *Ecotoxicology* 12:199–208
799. Franceschini MD, Custer CM, Custer TW, Reed JM, Romero LM 2008 Corticosterone stress response in tree swallows nesting near polychlorinated biphenyl- and dioxin-contaminated rivers. *Environ Toxicol Chem* 27:2326–2331
800. Axelstad M, Boberg J, Hougaard KS, Christiansen S, Jacobsen PR, Mandrup KR, Nellemann C, Lund SP, Hass U 2011 Effects of pre- and postnatal exposure to the UV-filter octyl methoxycinnamate (OMC) on the reproductive, auditory and neurological development of rat offspring. *Toxicol Appl Pharmacol* 250:278–290
801. Riegel AC, French ED 1999 Acute toluene induces biphasic changes in rat spontaneous locomotor activity which are blocked by remoxipride. *Pharmacol Biochem Behav* 62:399–402
802. Fan F, Wierda D, Rozman KK 1996 Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on humoral and cell-mediated immunity in Sprague-Dawley rats. *Toxicology* 106:221–228
803. Teeguarden JG, Dragan YP, Singh J, Vaughan J, Xu YH, Goldsworthy T, Pitot HC 1999 Quantitative analysis of dose- and time-dependent promotion of four phenotypes of altered hepatic foci by 2,3,7,8-tetrachlorodibenzo-*p*-di-

- oxin in female Sprague-Dawley rats. *Toxicol Sci* 51:211–223
804. Höfer N, Diel P, Wittsiede J, Wilhelm M, Kluxen FM, Degen GH 2010 Investigations on the estrogenic activity of the metallothionein cadmium in the rat intestine. *Arch Toxicol* 84:541–552
805. Zhang Y, Shen G, Yu Y, Zhu H 2009 The hormetic effect of cadmium on the activity of antioxidant enzymes in the earthworm *Eisenia fetida*. *Environ Pollut* 157:3064–3068
806. Sharma B, Patiño R 2009 Effects of cadmium on growth, metamorphosis and gonadal sex differentiation in tadpoles of the African clawed frog, *Xenopus laevis*. *Chemosphere* 76:1048–1055
807. Wang CR, Tian Y, Wang XR, Yu HX, Lu XW, Wang C, Wang H 2010 Hormesis effects and implicative application in assessment of lead-contaminated soils in roots of *Vicia faba* seedlings. *Chemosphere* 80:965–971
808. Fox DA, Kala SV, Hamilton WR, Johnson JE, O'Callaghan JP 2008 Low-level human equivalent gestational lead exposure produces supernormal scotopic electroretinograms, increased retinal neurogenesis, and decreased retinal dopamine utilization in rats. *Environ Health Perspect* 116:618–625
809. Chiang EC, Shen S, Kengeri SS, Xu H, Combs GF, Morris JS, Bostwick DG, Waters DJ 2009 Defining the optimal selenium dose for prostate cancer risk reduction: insights from the U-shaped relationship between selenium status, DNA damage, and apoptosis. *Dose Response* 8:285–300
810. Harding LE 2008 Non-linear uptake and hormesis effects of selenium in red-winged blackbirds (*Agelaius phoeniceus*). *Sci Total Environ* 389:350–366
811. Wisniewski AB, Cernetich A, Gearhart JP, Klein SL 2005 Perinatal exposure to genistein alters reproductive development and aggressive behavior in male mice. *Physiol Behav* 84:327–334
812. Anderson JJ, Ambrose WW, Garner SC 1998 Biphasic effects of genistein on bone tissue in the ovariectomized, lactating rat model. *Proc Soc Exp Biol Med* 217:345–350
813. Dey A, Guha P, Chattopadhyay S, Bandyopadhyay SK 2009 Biphasic activity of resveratrol on indomethacin-induced gastric ulcers. *Biochem Biophys Res Commun* 381:90–95
814. Boccia MM, Kopf SR, Baratti CM 1999 Phlorizin, a competitive inhibitor of glucose transport, facilitates memory storage in mice. *Neurobiol Learn Mem* 71:104–112
815. Brodeur JC, Svartz G, Perez-Coll CS, Marino DJ, Herkovits J 2009 Comparative susceptibility to atrazine of three developmental stages of *Rhinella arenarum* and influence on metamorphosis: non-monotonous acceleration of the time to climax and delayed tail resorption. *Aquat Toxicol* 91:161–170
816. Freeman JL, Beccue N, Rayburn AL 2005 Differential metamorphosis alters the endocrine response in anuran larvae exposed to T3 and atrazine. *Aquat Toxicol* 75:263–276
817. Undeđer U, Schlumpf M, Lichtensteiger W 2010 Effect of the herbicide pendimethalin on rat uterine weight and gene expression and in silico receptor binding analysis. *Food Chem Toxicol* 48:502–508
818. Cavieres MF, Jaeger J, Porter W 2002 Developmental toxicity of a commercial herbicide mixture in mice. I. Effects on embryo implantation and litter size. *Environ Health Perspect* 110:1081–1085
819. Zorrilla LM, Gibson EK, Stoker TE 2010 The effects of simazine, a chlorotriazine herbicide, on pubertal development in the female Wistar rat. *Reprod Toxicol* 29:393–400
820. Bloomquist JR, Barlow RL, Gillette JS, Li W, Kirby ML 2002 Selective effects of insecticides on nigrostriatal dopaminergic nerve pathways. *Neurotoxicology* 23:537–544
821. Lassiter TL, Brimijoin S 2008 Rats gain excess weight after developmental exposure to the organophosphorothionate pesticide, chlorpyrifos. *Neurotoxicol Teratol* 30:125–130
822. Wu H, Zhang R, Liu J, Guo Y, Ma E 2011 Effects of malathion and chlorpyrifos on acetylcholinesterase and antioxidant defense system in *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae). *Chemosphere* 83:599–604
823. Muthuviveganandavel V, Muthuraman P, Muthu S, Sri-kumar K 2008 Toxic effects of carbendazim at low dose levels in male rats. *J Toxicol Sci* 33:25–30
824. Laughlin GA, Goodell V, Barrett-Connor E 2010 Extremes of endogenous testosterone are associated with increased risk of incident coronary events in older women. *J Clin Endocrinol Metab* 95:740–747
825. Kratzik CW, Schatzl G, Lackner JE, Lunglmayr G, Brandstätter N, Rücklinger E, Huber J 2007 Mood changes, body mass index and bioavailable testosterone in healthy men: results of the Androx Vienna Municipality Study. *BJU Int* 100:614–618
826. Floege J, Kim J, Ireland E, Chazot C, Druke T, de Francisco A, Kronenberg F, Marcelli D, Passlick-Deetjen J, Scherthaner G, Fouqueray B, Wheeler DC 2010 Serum iPTH, calcium and phosphate, and the risk of mortality in a European haemodialysis population. *Nephrol Dial Transplant* 26:1948–1955
827. Danese MD, Kim J, Doan QV, Dylan M, Griffiths R, Chertow GM 2006 PTH and the risks for hip, vertebral, and pelvic fractures among patients on dialysis. *Am J Kidney Dis* 47:149–156
828. Tan ZS, Beiser A, Vasan RS, Au R, Auerbach S, Kiel DP, Wolf PA, Seshadri S 2008 Thyroid function and the risk of Alzheimer disease: the Framingham Study. *Arch Intern Med* 168:1514–1520
829. Tanaka M, Fukui M, Tomiyasu K, Akabame S, Nakano K, Hasegawa G, Oda Y, Nakamura N 2010 U-shaped relationship between insulin level and coronary artery calcification (CAC). *J Atheroscler Thromb* 17:1033–1040
830. Pyörälä M, Miettinen H, Laakso M, Pyörälä K 2000 Plasma insulin and all-cause, cardiovascular, and noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study. *Diabetes Care* 23:1097–1102
831. Kumari M, Chandola T, Brunner E, Kivimaki M 2010 A nonlinear relationship of generalized and central obesity with diurnal cortisol secretion in the Whitehall II study. *J Clin Endocrinol Metab* 95:4415–4423
832. Bremner MA, Deeg DJ, Beekman AT, Penninx BW, Lips P, Hoogendijk WJ 2007 Major depression in late life is associated with both hypo- and hypercortisolemia. *Biol Psychiatry* 62:479–486
833. Lee DH, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs Jr DR 2010 Low dose of some persistent organic

- pollutants predicts type 2 diabetes: a nested case-control study. *Environ Health Perspect* 118:1235–1242
834. Mendez MA, Garcia-Esteban R, Guxens M, Vrijheid M, Kogevinas M, Goñi F, Fochs S, Sunyer J 2011 Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. *Environ Health Perspect* 119:272–278
835. Cho MR, Shin JY, Hwang JH, Jacobs DR Jr, Kim SY, Lee DH 2011 Associations of fat mass and lean mass with bone mineral density differ by levels of persistent organic pollutants: National Health and Nutrition Examination Survey 1999–2004. *Chemosphere* 82:1268–1276
836. Monica Lind P, Lind L 10 May 2011 Circulating levels of bisphenol A and phthalates are related to carotid atherosclerosis in the elderly. *Atherosclerosis* 10.1016/j.atherosclerosis.2011.1005.1001
837. Melzer D, Rice N, Depledge MH, Henley WE, Galloway TS 2010 Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. *Environ Health Perspect* 118:686–692
838. Trabert B, De Roos AJ, Schwartz SM, Peters U, Scholes D, Barr DB, Holt VL 2010 Non-dioxin-like polychlorinated biphenyls and risk of endometriosis. *Environ Health Perspect* 118:1280–1285
839. Kim KY, Kim DS, Lee SK, Lee IK, Kang JH, Chang YS, Jacobs DR, Steffes M, Lee DH 2010 Association of low-dose exposure to persistent organic pollutants with global DNA hypomethylation in healthy Koreans. *Environ Health Perspect* 118:370–374
840. Laclaustra M, Navas-Acien A, Stranges S, Ordovas JM, Guallar E 2009 Serum selenium concentrations and diabetes in U.S. adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. *Atherosclerosis* 117:1409–1413
841. Laclaustra M, Stranges S, Navas-Acien A, Ordovas JM, Guallar E 2010 Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. *Atherosclerosis* 210:643–648
842. Ahmed S, Mahabbat-e Khoda S, Rekha RS, Gardner RM, Ameer SS, Moore S, Ekström EC, Vahter M, Raqib R 2011 Arsenic-associated oxidative stress, inflammation, and immune disruption in human placenta and cord blood. *Environ Health Perspect* 119:258–264
843. Claus Henn B, Ettinger AS, Schwartz J, Téllez-Rojo MM, Lamadrid-Figueroa H, Hernández-Avila M, Schnaas L, Amarasiriwardena C, Bellinger DC, Hu H, Wright RO 2010 Early postnatal blood manganese levels and children's neurodevelopment. *Epidemiology* 21:433–439
844. Wirth JJ, Rossano MG, Daly DC, Paneth N, Puscheck E, Potter RC, Diamond MP 2007 Ambient manganese exposure is negatively associated with human sperm motility and concentration. *Epidemiology* 18:270–273
845. Lee DH, Lee IK, Porta M, Steffes M, Jacobs Jr DR 2007 Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002. *Diabetologia* 50:1841–1851