Journal of Hazardous Materials 286 (2015) 589–611

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Review

Why endocrine disrupting chemicals (EDCs) challenge traditional risk assessment and how to respond

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HIGHLIGHTS

- Endocrine disrupting chemicals (EDCs) considered in relation to traditional risk assessment.
- Many characteristics pronounced in EDCs challenge traditional risk assessment.
- Human health can be best protected with a future risk framework tailored to EDCs.
- For now EDC risk may be transparently assessed with available tools and methods.

ARTICLE INFO

Article history:
Received 24 June 2014
Received in revised form 2 December 2014
Accepted 8 December 2014
Available online 12 December 2014

Keywords:
Risk assessment
Endocrine disrupting chemical
Human health
Hormonally active agent
Microcontaminant

ABSTRACT

Endocrine disrupting compounds (EDCs) are a diverse group of "chemicals of emerging concern" which have attracted much interest from the research community since the 1990s. Today there is still no definitive risk assessment tool for EDCs. While some decision making organizations have attempted to design methodology guidelines to evaluate the potential risk from this broadly defined group of constituents, risk assessors still face many uncertainties and unknowns. Until a risk assessment paradigm is designed specifically for EDCs and is vetted by the field, traditional risk assessment tools may be used with caution to evaluate EDCs. In doing so, each issue of contention should be addressed with transparency in order to leverage available information and technology without sacrificing integrity or accuracy. The challenges that EDCs pose to traditional risk assessment are described in this article to assist in this process.

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http://dx.doi.org/10.1016/j.jhazmat.2014.12.012
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1. Introduction

More than at any other time risk assessment is at a crossroads—one path leads to retaining the status quo the other to a challenging but less certain future.

Let's hope we have the wisdom to choose correctly. [56].

"Chemicals of emerging concern" have attracted much attention in the last half decade [1–8]. One group among them, endocrine disrupting chemicals (EDCs)—also known as endocrine disrupting compounds, endocrine disruptor contaminants, hormonally active agents, and endocrine active substances—make headlines regularly [9–12]. Broadly speaking, EDCs are substances that influence processes associated with the endocrine system and alter its functioning [13,14]. The endocrine system is responsible for the release of hormones, chemicals which help control and connect the systems of the body, and thus plays a major role in the health of humans and wildlife [15]. EDCs interfere with steroids such as estrogen, anti-androgen and androgen, as well as other hormone action [14].

As the endocrine system is tightly regulated during certain life stages, small changes in hormonal conditions, even for a brief time, can have acute and enduring impacts on exposed populations [16]. According to the World Health Organization (WHO) and the Organisation for Economic Co-Operation and Development (OECD), changes to natural hormonal functions (i.e., synthesis, secretion, transport, binding, action, elimination) [16] can cause adverse health effects in an intact organism, or its progeny or (sub) populations, consequent to changes in endocrine function [17,18]. Though the term EDC is often thought of as referring only to synthetic chemicals, natural agents can also be endocrine-active [19]. EDCs can be grouped in many ways, by structure or function (see Appendix B for examples of EDC categories). For example, estrogenic EDCs produced naturally by plants are called phytoestrogens (e.g., isoflavones), and synthetically are called xenoestrogens (e.g., phthalates) [20,21]. A large portion of identified EDCs are xenoestrogens [22], although their precise number in the ecosystem is unknown [23].

The term "endocrine disruptor" came into use in 1991 after a working session on chemically-induced alternations in sexual development as part of the Wingspread Conference [24]. This was the first time a group of researchers reached a public consensus that endocrine disrupting chemicals in the environment were disturbing reproductive health [25,26]. Five years later, a book called "Our Stolen Future" [27] brought attention to the widespread ecological and human health consequences of EDCs. These events sparked a wave of research, and brought to light the high potential for risk to human health posed by EDC exposure [28,29]. Still, progress in developing the method for systematic assessment of associated risks from these chemicals has been only modest [30] relative to methods for quantifying other types of health threats such as carcinogens [31,32] and natural hazards [33,34].

It is proposed here that risk assessment (RA) of EDCs should be undertaken promptly by leveraging available methods and analytical tools, but should be done with caution and should be accompanied by appropriate risk communication. This will help keep provisional results from being misconstrued as definitive risk values, and resulting in unwise decisions and unsafe situation. Subsequently, comprehensive guidelines should be developed specifically for EDC risk assessment in which the unique characteristics of this chemical group are addressed. Table 1 lists some of these challenges which make conventional risk assessment complicated or ill-suited for application to EDC human health risk.

There are also some challenges to exposure assessment and RA that, while not unique to EDCs, are prominent in this chemical group and worth noting. These are discussed in Appendix A:

- EDCs have been transported all over the globe and are ubiquitous in the environment,
- Effective exposure to EDCs is complex to calculate,
- Limitations in epidemiological and toxicological study limit reliable data for RA, and

Table 1

Challenges Posed by EDCs to Traditional Risk Assessment.
Lack of a universal definition of EDC
Incomplete data on most EDCs and potential EDCs
Humans have long-term exposure to a combination of EDCs
EDC's exposure regimes are fundamentally different from traditional chemicals
Timing of exposure can be as important as dose size for EDCs
EDCs often display latent transgenerational effects
Gaps in knowledge exist regarding mechanism and mode of action for EDCs
EDC effects are not always categorically adverse, and "adversity" is not universally defined
No consensus on what endpoint(s) are best to use in EDC toxicological studies
EDC mixtures can have unknown combination effects (i.e., additive, synergistic)
EDCs can show low dose effects at miniscule concentrations
Some EDCs have non-monotonic dose–response curves which are less compatible with traditional risk assessment
EDCs in background environment threatens to contaminate study results
EDCs may not have a "safe" threshold for doses linked to detrimental effects
EDC uncertainties/unknowns hinder the accurate calculation of absolute risk (but permit assessment of relative risk)
EDC risk from a given source should be understood in context, in relation to: other sources, improving detection, and what is tolerable by society
Appropriate communication of EDC risk findings needs to balance precaution and alarm

- Extrapolations between EDC studies at different levels of organization (i.e., cell-to-human, inter-species, sub-chronic-to-chronic/long-term) introduce uncertainty.

This article will be the first to systematically and comprehensively detail these obstacles that EDCs pose to traditional risk assessment, and how they can be tempered or overcome. This is an important step in modifying human health risk assessment methodology specifically for EDCs so it can best aid decision makers in responding to EDC exposure. This tailored EDC RA approach should be honed gradually, but no time should be lost in its application; despite the complications, current RA tools used properly can inform EDC risk.

2. Challenges posed by EDCs to traditional RA

There are five basic stages in analyzing and addressing risk [16,35–37], as demonstrated in Fig. 1. Risk is generally defined as the product of hazard and exposure [38], taking into account uncertainty. Many contemporary RAs are based on the handful of early keystone frameworks such as the report "Risk assessment in the Federal Government: Managing the process," also called the "Red Book" by the United States National Research Council (US NRC) [36,39], and the NRC's more recent "Science and Decisions: Advancing Risk Assessment" referred to as the "Silver Book" [37]. Other key RA publications include the United States Environmental Protection Agency's (EPA) "Risk Assessment and Management: Framework for Decision Making [40]; the redefined US NRC framework [41]; the European Union (EU) risk assessment framework for industrial chemicals [42]; and the framework for risk management and risk assessment of food additives developed by the Food and Agricultural Organization (FAO) and the WHO [43]. Today, steps have been taken by the WHO International Programme on Chemical Safety (WHO/IPCS), in collaboration with the EPA and the Organization of Economic Co-operation and Development (OECD) in developing an integrated framework for both human health and the environment [36,44–47]. For more than a decade RA methodology that informs regulation has been continually modified [48].

Guidelines for evaluating risk of EDCs in the environment have been relatively uncoordinated, and EDCs are not yet addressed comprehensively under existing regulations [49,50]. However, some decision making organizations have attempted to design methodology guidelines to evaluate the potential risk from this broadly defined group of constituents. These include government agencies in Australia [51,52], the EU [42,53,54], and the US EPA [55]. However, risk assessors still face many uncertainties and unknowns with regards to EDCs. The best methods for applying scientific information to human health RA remain under discussion internationally [56].

A detailed, systematic, standardized risk assessment paradigm for EDCs has not been established due to the associated scientific ambiguities, testing complexities and administrative hurdles [38,57–59]. To fully understand the reasons for the slow progress, it is important to consider the ways in which EDCs do not obey traditional principles of toxicology [56,60,61]. These areas of uncertainty and gaps in knowledge are described below in Sections 2.1–2.4, sorted according to the RA step they most affect, though in fact there is great overlap between the categories.

2.1. Hazard characterization

2.1.1. EDC definition

At present, there is still no permanent consensus on what precisely constitutes an EDC [49,62,63] which makes it difficult or misleading to comment on the available methods for EDC RA (or lack thereof). Some stipulate that the definition for an EDC requires two elements: "that of the demonstration of an adverse effect and of an endocrine disrupting mode-of-action" [64]. This approach is consistent with the idea that EDCs cannot always be identified by structure [23,65,66]; each needs to be tested for their unique endocrine effects [57,63]. This constitutes a massive undertaking considering that EDCs make up such a highly heterogeneous chemical group. Today much is yet unknown about how EDCs function, making it difficult to predict in advance whether a given compound will exert endocrine-disrupting properties [28].

Precise terminology and definitions are important when dealing with risk [67], especially when considering their potential impact on future regulation. For example, the EPA defines EDCs as an "exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior," [68] which is adopted from Kavlock et al. [16]. This definition is without reference to the quality of the interference. The Endocrine Society supports a similar definition that intentionally omits reference to "adverse" effects because the nature of a chemical's interference with hormone action depends in part on the timing of exposure [69].

WHO/IPCS provided the most recent working definition of EDCs [70], which is widely accepted and functional in the context of environmental and human risk assessment [71,72]: "An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) pop-

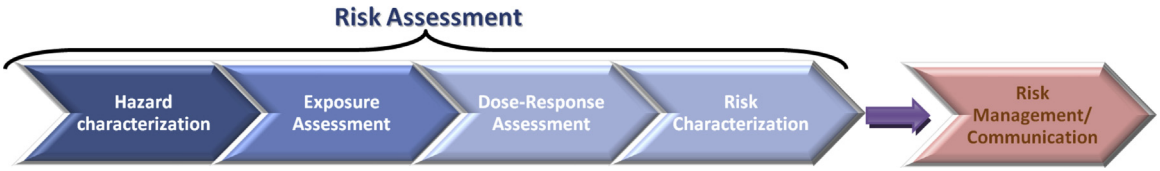

Fig. 1. Stages of risk assessment and management.

ulations" [18]. This definition does include the word "adverse," consequently limiting the scope of chemicals included. Unlike the EPA which chose not to specifically incorporate the opinion of outside stakeholders in its definition, the WHO defined EDCs via a committee that included industry representatives [73]. The stakeholders on the WHO committee arguably benefited from casting a narrower net in categorizing EDCs, as fewer chemicals under regulation would mean fewer risk assessments and restrictions on their products. Seeing precision as an advantage, the EC and the European Food Safety Authority adopted this WHO definition [57,74].

Although this disagreement over the lack of a universal EDC definition makes hazard characterization for an RA more difficult, efforts in the EU and US are underway to clarify the definition, better characterize its boundaries, and establish a definition that is scientifically accurate yet also helpful from a regulatory perspective [57,69,75]. For example, the minutes from a recent European Commission (EC) expert meeting on endocrine disruptors state that: "participants quickly agreed that there is no major controversy in science around the definition of endocrine disrupting chemicals. All can agree upon the definition provided by the WHO, which is also used by EFSA. Substances can act either directly or indirectly on the endocrine system, which are being discussed as part of the criteria, but this does not affect the baseline definition of EDCs" [74]. Organizations such as the EPA will have to weigh any concern over this definition against the benefits of global consensus.

2.1.2. Incomplete data

Hundreds of chemicals have been labeled EDCs, but the vast majority of chemicals in current use have not been tested at all [29]. The EPA estimates that approximately 87,000 chemicals will merit screening for endocrine potential [76], and there are countless more potential EDCs being discovered and explored every year [27]. For example, a federal agency collaboration in the US called Tox21 will soon screen a list of over 10,000 chemicals for endocrine disruption which will likely identify many new potential EDCs [77]. The scale of the research needs in this area pushes the resource and technological limits of government and professional organizations alike [23].

The stock of EDC information needed to run an accurate RA is incomplete but growing. As it accumulates, EDC study results should be pooled in a single unified database for public accessibility [78], convenience, and cooperation [29]. Sharing information would help avoid redundant research and wasted resources. This kind of cooperation that combines resources would benefit all parties, and would collectively add up to more than the sum of its parts. Today, the databases outlined in Table 2 provide the public with information on EDC.

2.2. Exposure assessment

2.2.1. Long-term and combined exposure

As EDCs are ubiquitous in today's world [87,88], subchronic and chronic exposure at low doses over time is common [29]. Combined EDC exposure from a variety of routes may approach or exceed so-called "safe" doses [89] complicating efforts to establish a discrete exposure level for use in RA. Experimental studies have already shown indications of risk specific to longer-term exposure [74]. Therefore, notwithstanding the significant cost and labor, more long-term research would be beneficial whenever circumstances allow. This should be made a priority by EDC research and testing programs including the EPA's Endocrine Disruptor Screening program and ECHA's (the European Chemical Agency) REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulation in the EU.

There are few established health criteria for long-term low-level environmental exposure to EDCs [90]. Researchers are working to determine exposure levels that are unlikely to be associated with adverse health effects assuming daily exposure over an extended period of time. Several agencies have standard methods for doing so: World Health Organization's Tolerable Daily Intake or TDI [91], US Environmental Protection Agency's Reference Dose [55,92], US Department of Health and Human Services' Agency for Toxic Substances and Disease Registry's Minimum risk level [93], or the EU's Risk quotient [53]. Progress in these efforts is likely to improve the precision and replicability of RA for EDCs in the future.

2.2.2. Unique exposure regimes

The exposure profile for EDCs is broad and potentially complicated as anthropogenic sources include agricultural, industrial, domestic and pharmaceutical applications [87,94–98]. Moreover, human exposure regimes of many EDCs differ from more traditional contaminants [89]. For example, in addition to incidental, accidental or otherwise involuntary exposure, adults can be exposed to high levels of EDCs through deliberate action such as taking medicine or using consumer products [89]. Then, as mentioned, contact with multiple EDCs that have similar targets can result in combined exposure that poses a threat to health where individual exposures do not [89,99].

Table 2
Sample EDC databases.

Organization	Content	Citation
European Commission	Database and reporting	[79]
US Environmental Protection Agency	ToxCast	[80]
US Food and Drug Administration	Endocrine Disruptor Knowledge Base	[81]
The European Chemicals Agency	ECHA chemical database	[82]
WHO/United Nations Environmental Programme	Report on the State of Science of EDCs	[29]
Thomas Hartung, MD PhD (Principal investigator)	The Human Toxome Project	[83]
US National Library of Medicine	Environmental Health and Toxicology Portal	[84]
Istituto Superiore di Sanita (ISS)	EDCs Diet Interaction Database	[85]
The Endocrine Disruption Exchange (TEDX)	List of Potential Endocrine Disruptors	[86]

Another important difference is that while infants are generally exposed less than adults to traditional chemicals, this is not necessarily the case with EDCs. .Endocrine disrupting chemicals have been found in materials intended for use by infants such as flame retardant pajamas and baby bottles, as well as in food, formula and breast milk [89,100]. Young children can have higher exposure to chemicals due to higher metabolic rates; more ingestion of food, drink and air per body weight compared to adults; higher hand-to-mouth and object-to-mouth activity; and high rates of development susceptible to environmental contaminants [101,102].

These differences suggest larger information gaps in the classification of exposure to EDCs, not only by age group but also gender, source, pathway, and potency [60]. Large scale monitoring networks with progressively better temporal and spatial resolution can help estimate exposure more accurately and at less cost. These are increasingly aided by technological advances such as biosensors for estrogenic activity [146]. Monitoring programs can help map cumulative exposure from a variety of sources, and biosensors can help estimate average individual exposure for different segments of the population including those that are most vulnerable.

2.2.3. Timing

When calculating exposure to EDCs for RA, some key temporal characteristics should be considered; EDC exposure is life stage and timing dependent [15]. As with other reproductive and developmental toxins, there are cases in which timing of the dose is more important than the magnitude of the dose since sensitivity to exposure is heightened during critical developmental periods [19,103]. Since hormones regulate functions in the body through very precise amounts of chemicals released at very specific times, EDC effects are amplified during these "hormone-driven stages" [104–111]. Windows of vulnerability include in utero, infancy, childhood, puberty and menopause [74], and the effects of early life exposure can create ripple effects that manifest across different areas of development in later life [28,112,113].

Moreover, it has been shown that EDC exposure in pre-birth or early life may sometimes manifest only later in life or even future generations [114,115]. There is evidence that embryonic, fetal, and neonatal tissues may interpret estrogens and EDCs in a different way — or by different mechanisms — than adult tissues [16]. However, as few studies evaluate the late-life effects of prenatal or early life exposure, such a distant connection between exposure and effect may not be captured by traditional risk assessment. More long-term work on EDCs is needed, alongside studies of latent or adulthood effects of exposure during development [72]. Already, multigenerational tests are being developed internationally and vetted in the US [116].

2.2.4. Transgenerational effects

A temporal component of EDC exposure and impact related to EDC persistence over generations is called the transgenerational effect [117–119]. The transgenerational nature of EDC effects is due in part to their persistence. Many EDCs have long half-lives and are resistant to degradation in the environment. These include polychlorinated biphenyls (PCBs), nonylphenol, tributyltin, and polychlorinated dibenzofurans (PCDFs) [28,120,121].

Highly persistent synthetic EDCs are sometimes lipophilic (fat soluble) [122], such as polychlorinated dibenzodioxins. These compounds can undergo bioaccumulation, a process in which they are stored and amassed in the body [120,123–127] within adipose tissue [128,129]. These EDCs move up through the food chain as predators consume the fat of their prey [28] which increases their concentrations in keystone species such as bald eagles [28,130], polar bears [27], and ultimately humans at the top of the food chain. Humans can pass their legacy of accumulated EDCs to offspring in breast milk [100,131–134].

The rarer temporal characteristic which poses a challenge to traditional risk assessment, however, is that the effects of certain EDCs can be passed down in humans. This can occur either through genotoxicity in which heritable damage occurs to DNA [21,135] similar to radiation exposure, or through epigenetic impacts in which changes are transmitted by affecting the way genes are turned on and off [117–119,136]. This adds to the extended latency of effects from exposure during vulnerable pre-maturity windows, and makes it all the more difficult to link exposure to impacts [118,137]. It took many years for the health implications of EDCs to be recognized and regulatory responses considered because their most devastating consequences are sometimes manifested only generations later, in the offspring of those exposed [138,139]. To accommodate the temporal dimensions of EDC exposure, multigenerational studies would need to investigate a range of dosage levels at different life stages [140].

2.3. Dose–response assessment

2.3.1. Mechanism and mode of action

A chemical's mode of action (MOA) is described as "the biologically plausible sequence of key events, starting with the interaction of an agent with a cell, through functional and anatomical changes, leading to an observed effect supported by robust experimental observations and mechanistic data" [141]. A chemical's mechanism of action describes the biochemical interaction taking place, usually at the cellular level, through which it produces an effect on the body. Often mode and mechanism are used interchangeably in this context, but sometimes they are considered different in that "the latter implies a more detailed understanding of the molecular basis of the toxic effect" [142]. EDCs can have complex modes of action with multiple components that manifest uniquely within a given species [60,143,144] and the effect an EDC on different species may vary [144]. This makes it difficult to extrapolate information from one species to another, or between levels of organization (e.g., cell, organ, organism).

Adding to the complication surrounding RA of EDCs is the fact that crosstalk among several mechanisms and modes of action—or between pathways— can take place [30]. EDCs can affect the endocrine system at many different locations [145], and perturbations can influence an entire web of processes responsible for reproduction, such as development, behavior, and homeostasis maintenance [13,14,119,146–148]. Problems regulating each of these interconnected systems and body parts can have ripple effects among the entire network of processes [127]. EDC exposure can therefore lead to a wide variety of intertwined health and behavioral consequences [28,149].

Drawing individual direct lines between parts and functions affected by EDCs oversimplifies an extremely complicated process [60]. Researchers cannot always attribute causation and must interpret results with caution because of the vastness of the EDC field, and due to definitional and methodological questions [150]. Sometimes a biologically plausible linkage between an EDC and an observed adverse effect indicates causation, with the amount of information needed as evidence depending on the type of effect [70]. To help map and explore individual mechanism and MOA pathways and the linkages between them, a tool called Adverse Outcome Pathway frameworks has been developed. Its use in RA is currently being refined [116], and it will be further discussed below in Section 3.5, "Advancements in RA of EDCs."

In addition, compiling mechanism and mode information on EDCs in a global database will help create categories within which available information is generalizable. While not a definitive indication of mechanism, these categories may hint at the functioning

of EDCs (or potential EDCs) that has not been yet tested, but which structurally resemble other well-characterized chemicals in a group. To assist in this process, Quantitative Structure Activity Relationship (QSAR) modeling can help identify chemicals that are not a good fit for specific subgroups of chemicals according to structure [151].

2.3.2. Adversity

Even when mechanisms, modes of action and extrapolation are relatively well-established, EDCs can display characteristics that do not fit comfortably into traditional RA frameworks; their effects are not always definitively adverse as would be expected from a traditional toxin [62]. Some chemicals influence the endocrine system in a way that has a minimal or arguably benign effect to which the body has no trouble adapting. Often, these chemicals act alongside or instead of endogenous chemicals such as hormones. To complicate matters, sometimes there is no effect at high levels but adversity at low levels, as well as vice versa.

This issue is exacerbated by disagreement over a more fundamental point: how to define the word "adverse" [15,36,57,152]. The EPA defines it as "a biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge" [153]. The definition commonly used in the international community in relation to endocrine action is from the WHO's International Programme on Chemical Safety (IPCS): "A change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences" [154]. The US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) do not define adverse [155].

This widely accepted WHO definition of adverse effects describes "a change," but it does not make a clear distinction between a harmful reaction and an adaptive one. In a 2011 workshop held by the Health and Environmental Sciences Institute (HESI) committee adopted the WHO definition, and added the following to help distinguish it from effects that produced adaptive endocrine responses: "Adaptive Response [is defined as]: in the context of toxicology, the process whereby a cell or organism responds to a xenobiotic so that the cell or organism will survive in the new environment that contains the xenobiotic without impairment of function" [152]. So it seems that the definition of "adaptive" responses is no impairment of function while "aversive" implies compromised function or impairment of ability to respond to additional stress. Improving the precision of language used in this field continues to help the risk assessment community focus on priority areas and the most potentially harmful EDCs.

2.3.3. Endpoints

Another basic but unresolved question involves what toxicological data risk assessors of EDCs should be using to determine adversity and risk from EDCs in living organisms [155]. This boils down to the choice of an "endpoint," defined as an (adverse) effect on a human, animal, plant or other ecological population whose risk for harm is evaluated. In other words, it is the damaging end result of a perturbation. One chemical can have an array of effects depending on the receiver species, the exposure timing (see Section 2.2.3 above), and the mixture in which it was delivered (see Section 2.4.4 below). Generally the most sensitive adverse endpoint is chosen, but this is not always a clear-cut choice; there is no "gold standard" for such a determination [23].

Hormonally active agents exhibit a high endocrine disrupting potency for various endpoints [156]. EDC endpoints for human health RAs should be chosen with an eye to human relevance, ideally accompanied by a MOA [72]. For EDCs, endpoints mostly involve individuals' primary sexual characteristics related to reproduction such as anogenital distance [157], testicular descent, penile length and width, and scrotal condition [14]. Several non-reproductive EDC endpoints have also been reported [158] including hepatic and renal effects (DBP), hepatocellular carcinoma (DEHP), anovulation, and decreased fetal growth (DEHP) [159].

Toxicology studies in the past have usually relied on "apical" endpoints. Apical endpoints are "empirically verifiable outcomes of exposure, such as developmental anomalies, breeding behaviors, impaired reproduction, physical changes and alterations in the size and histopathology of organs, and, of course, death" [160]. Today there is a movement towards incorporating intermediate toxicological effects in RA by exploiting medium- and high-throughput in vitro testing to detect earlier "biological perturbations" [161], instead of relying solely on apical endpoints [152,160]. According to the National Research Council [161] these intermediate changes are "initial perturbations of cell-signaling motifs, genetic circuits, and cellular-response networks," and "are obligatory changes resulting from chemical exposure that might eventually result in disease" [160]. They occur further upstream than apical endpoints, so they can be more discreet and difficult to link to adverse outcomes—detection of in vitro endocrine disruption does not imply human health risk, nor does its absence promise safety [162]. The tradeoff is that they can help provide earlier and more fundamental indications of human health problems, and thus more accurate estimations of risk.

Going beyond apical endpoints to developing a richer understanding of full cellular pathways, especially those linked to adversity that can be used in RA, will ultimately help reduce animal testing and improve regulation [152]. To this end, two new terms were coined at the 2011HESI workshops (mentioned in Section 2.3.2) to help classify endpoints of concern: Relevant Pathways of Toxicological Concern (RPTCs), and Relevant Responses for Regulation (RRRs). RPTCs are "biochemical pathways associated with adverse events," and RRRs are "the endpoints forming the basis for RA and may or may not be at the level of the pathways" [152]. Improved understanding of RPTCs— quantitative descriptions, dose transition points, etc. with the help of computational models—along with detailed information on dosimetry, exposure, and time and dose continuums will decrease uncertainty and encourage selection of appropriate endpoints in RA [152].

It is not only difficult to know which endpoints are appropriate when testing a chemical, but also how many endpoints to use. If too few are utilized, then it is possible that an adverse effect will be missed. However, testing for too many endpoints is costly, and is statistically likely to yield random false positives when the number gets high enough. The increase in testing group sizes which can overcome this statistical phenomenon is usually prohibitively expensive [163]. To help with the endpoint selection process, validation of toxicology testing methods for key endocrine disrupting endpoints is currently underway [57,60,164,165].

2.3.4. Mixtures

There is a tendency to focus solely on the primary individual contributors in a RA even though environmental exposure usually involves multiple chemicals in EDC mixtures [51,60]. Mixture constituents can act via common modes of action [166–169], or by a variety of signal transduction pathways which might crosstalk [30] or produce other matrix effects [170,171]. "Combination effects" occur when synthetic EDCs interact with each other, or with natural compounds in the environment and in the body, and they can have additive, synergistic or attenuative potential [30,99,172].

When combined in a mixture, individual chemicals can contribute to toxicity in direct proportion to their potency and concentration, even if they are each present at a concentration

below their individual effect thresholds [99]. This phenomenon is termed "something from nothing" [173]. For example, exposure to various xenoestrogens can derail development in fish, even if the exposure for a single xenoestrogen is below the safe threshold level for the effects. As such, the real risk may be greater than that expected based on the effect of individual mixture components [174]. Experimental evidence shows that the cumulative impact of EDCs remains poorly understood [172,175,176], in part because traditional dose–response testing neglects the potential mixture effects not seen in single chemical testing.

Some types of EDCs require more sophisticated models that account for transformations and interactions with other chemicals [177]. To help address gaps in knowledge, there is a movement away from single "chemical by chemical" testing and risk assessment. The present trend prefers multi-chemical regulation of contaminant groups that share similar health effect endpoints, that are removed by relatively simple processes, and that can be measured by common analytical methods [178]. As such, this is particularly relevant for media that regularly undergo treatment, such as drinking water. By using a "relative potency factor" approach, the strength or toxicity for each chemical in the group is compared to an index chemical, exposures to the chemical group is combined and expressed as a total equivalent dose [178,179].

Only a better understanding of real-life combined exposures can determine whether risk is amplified by exposure to mixtures [99]. New information on EDC combination effects is being generated by new studies on chemical mixtures [174,180,181] and models that account for agonist and antagonist of hormonal activity [170]. More information is also needed regarding the toxicity of different parent compounds after they have broken down because metabolites and byproducts of chemical reactions can also be toxic [182], and can contribute to the potency of a mixture.

2.3.5. Low dose effects

Until the 1990's, it was believed that EDCs presented a risk only when exposure involved high doses. More recently it was found that endocrine systems can exhibit surprising potency at extremely low doses [172,183,184], such as in the case of steroidal estrogens [185]. Studies confirmed that EDCs can be hormonally active at minute concentrations: parts per billion, or even parts per trillion [15,50,146]. This is because of the nonlinear relationships between hormone concentration and receptor occupancy, and between receptor occupancy and biological effect [15]. Accordingly, the binding of an EDC to a hormone receptor can lead to an amplified response in the target cells even without a high rate of receptor occupancy [183,186]. This means that a minor change in the EDC concentration, even when this dose is small, can lead to significant changes in biological endpoints [15].

A scientific consensus regarding the biological plausibility of low dose effects will contribute to the establishment of a universally accepted RA methodology for EDCs [60]. This is hindered by the fact that "low dose" is not universally defined [184,187]. One broad definition, often used is from the 2000 EPA/NIEHS Endocrine Disruptor Low Dose Peer Review Workshop: "a biological change, not limited to adverse effects, which occur either at human exposure levels or at doses below those routinely used in toxicity testing" [188]. Based on this definition, data from new EDC toxicity studies show low dose perturbations at levels below those previously established to have no effect [56,60].

Evidence of low dose effects has in fact been collected for more than two dozen EDCs [149]. Despite some disagreement over individual EDCs such as BPA [15], most EDCs are deemed likely to have low dose effects for at least some endocrine-disrupting endpoints [146,149]. Only a small percentage of all EDCs have been tested for low dose effects on human health endpoints [15], so it is possible that chemicals deemed "safe" may pose previously unrecognized dangers.

As EDCs show effects at low doses not predicted by effects at higher doses, the traditional toxicological conception that "the dose makes the poison" is called into question [149]. The implications could be enormous as humans are chronically exposed to low doses of EDCs, often from non-point sources, making exposure difficult to calculate or control. Low doses of EDCs can be found in common products that see extensive use, ranging from plastics, fire retardants, sunscreen, and cosmetics, to medical equipment and even food [28,189–195]. More information is needed to understand the significance of low dose effects [56,187], and how this impacts risk assessment [196].

Organizations concerned with improving risk assessment are currently formulating research priorities for low dose effects at workshops in the US and the EU [188]. At a recent meeting in Berlin, it was established that more workshops, more literature reviews and improved communication between relevant parties were needed, especially between risk assessors and academics [56]. One speaker at this event [197] pointed out that the goals of regulatory toxicity studies often do not include establishing dose–response relationships: they do not include enough doses to characterize the relationship, and those included are too widely spaced. This speaker made the recommendation that in regulatory toxicity studies, identification of significant effects for specific endpoints at "intermediate" or "low" concentrations merit additional focused investigation. This means testing additional doses to characterize the dose–response relationship of observed effects.

2.3.6. Non-monotonic dose response curves

One line of low-dose–response research focuses on a phenomenon termed the "non-monotonic dose" response. Typical linear or threshold dose–response relations are those in which the frequency or severity of an effect increases as the dose increases. The relationship is reflected in ascending response curves that do not change direction (i.e., are monotonic). This coincides with the traditional toxicological paradigm in which a lower dose induces a smaller reaction. In contrast, non-monotonic dose–response curves (NMDRCs) contain at least one point in the tested dose range where the slope of the response curve changes directions. Whereas "low dose" is a relative term, NMDRCs are defined mathematically [15] and can yield inverted, U-shaped, or otherwise non-monotonic curves [15,149,198].

EDC research has uncovered many cases in which small doses of an EDC display greater potency than high doses in dose–response testing, resulting in a NMDRC. The mechanisms behind NMDRCs with EDCs occurrence include "cytotoxicity, cell and tissue-specific receptors and cofactors, receptor selectivity, receptor down-regulation and desensitization, receptor competition, and endocrine negative feedback loops" [155]. It is not always clear which specific mechanism is accountable for a given NMDRC [15]. However, in response to the publication of a two year study on EDC low dose effect and NMDRCs [149], Dr. Linda Birnbaum— Director of the National Institutes of Environmental Health Sciences— stated that "the question is no longer whether non-monotonic dose–responses are 'real' and occur frequently enough to be a concern; clearly these are common phenomena with well-understood mechanisms. Instead, the question is which dose–response shapes should be expected for specific environmental chemicals and under what specific circumstances" [199].

As Dr. Birnbaum stated, inverted U-shaped curves are not uncommon in the chemical universe, and usually do not pose a challenge to traditional RA, which is interested in the effects of low doses in the first part of the inverted U that is slanted upward. A NMDRC is of concern when it is U-shaped, and adverse effects occur at doses below those that would be identified as a "no observed

adverse effect level" (NOAEL) in a classical RA approach. In a traditional assessment of risk, the NOAEL is treated as the dose that does not pose harm. It is divided by uncertainty factors to arrive at a reference dose that accounts for things such as interspecies differences between the dose–response tests on animals and actual exposure scenarios involving humans. If there are NMDRC cases in which adverse effects in fact do occur at doses below the NOAEL, the RA will not capture risk accurately.

It has been suggested that low doses of some EDCs commonly induce effects displaying non-monotonic dose–response curves which would be missed in guideline-adhering toxicity studies because doses tested tend to significantly exceeded actual human exposure [15,140]. Low-dose extrapolation from higher doses could be inaccurate if the region on the curve below the NOAEL is actually non-monotonic. This brings into question the accuracy of chemical testing from which RA inputs are derived [15,140,149], a concern raised in a 2009 meeting on EDCs and human health organized by the German Federal Institute for Risk Assessment. Some work indicates that NMDRCs can affect RAs by limiting the usefulness of a common method (i.e., benchmark dose combined with the use of uncertainty factors) [72]. This has led to calls for fundamental changes in the ways chemicals are tested for human health safety [149]. Others are not convinced that NMDR impacts on RA are anticipated to result in mischaracterizations of a chemical using the current testing approaches [187,200].

Although the significance of NMDR findings in relation to characterization of the risk posed by EDCs is still under discussion by experts [56], there are some who feel that NMDRCs do not threaten the usefulness of traditional RA in most cases. For example, Dr. Earl Gray is a lead authority in this area working with the EPA, and his most recent observations are that:

- EDCs appear to induce some linear non-threshold effects;
- NMDRCs are biologically plausible and occur frequently in vitro, but often occur in vitro at high concentrations of estrogens or androgens that are not relevant in vivo;
- It appears that NMRDCs are more common in short- versus long-term exposures and with upstream, mechanistic events versus downstream phenotypic effects;
- A few adverse effects of EDs are non-monotonic, but other effects displaying monotonic responses occur at lower dosage levels;
- A number of robust multigenerational studies of estrogens and antiandrogens have been executed and NMDRCs were uncommon at low dosage levels;
- Multigenerational test guidelines can be enhanced on a case-by-case basis to improve the sensitivity to low dose effects of some EDCs; and
- Additional data need to be examined from robust, multigenerational studies using a broad range of dosage levels for other pathways [140].

An EPA draft report on NMDRs reflected the same conclusions [201], but a review the EPA commissioned by the National Academy of Sciences determined that the methodology behind this report lacked the necessary rigor, consistency, and replicability, and was thus "compromised." A new draft is forthcoming.

As with low dose effects, researchers seem to agree that more information is needed before the NMDRC controversy can be conclusively resolved [56,187]. The specific conditions for NMDRC occurrence are under debate at the EPA and were discussed in both national and international workshops [56,188]. Below are some of the critical questions being explored regarding an RA strategy for NMDRs [202]:

- In the world of chemicals how common are non-monotonic dose–response relationships?
- To what extent are currently recognized low dose effects clearly adverse and associated with human disease?
- How often are adverse, non-monotonic effects not covered by the standard defaults applied to NOEL values?
- Do current Thresholds of Toxicological Concern (TTC) values cover all monotonic dose effects?

Researchers are testing increasingly lower doses of EDCs to evaluate these questions with greater precision [185]. The computational modeling of NMDRCs is helping simplify this process [203]. It is important for scientists and journals to publish data demonstrating NMDRCs and low dose effects, even if the exact mechanism of action is not proven [149]. In the growing field of multidisciplinary research into EDCs, the more work available displaying these concepts, the sooner they will be understood.

2.3.7. Process contamination

When conducting tests on the effects of EDCs in which precise documentation of exposure is necessary, there are many ways in which the purity of an investigation can be jeopardized by background levels of EDCs. Many of these compounds are ubiquitous and contribute to background concentrations that introduce variability into study results. For example, in the past EDCs have been overlooked in products that are clear of traditional chemicals for lab use (e.g., plastic test tubes, animal cages, animal food, cleaning products) [27]. Even in a highly controlled lab environment, process contamination can be introduced by things as innocuous as a new rat cage, different bedding, a diet variation in calories or background levels of phytoestrogens, or a seasonal variation causing a change in endocrine reactions [184,204]. It is difficult to attain a pristine environment completely free of micropollutants in which to conduct experiments.

Such unexposed process contamination or design flaws may be behind some of the cases in which scientists have been unable to replicate results of EDC dose–response tests from previous studies [149,204]. Difficulty reproducing results not only occurs between different laboratories [205,206], but also within the same laboratory [207]. Specifically there has been controversy over the meaningfulness of NMDRCs and the lack of reproducibility of low dose effects [60,140,155,185,207,208], bringing into question the validity of the evidence and raising the possibility that initial results were flawed or biased. This has prompted some to question the true risk of exposure to some EDC [209,210], and whether the resources spent to study and regulate them are merited [211].

However, it should be noted that difficulty replicating results for EDCs does not necessarily mean the same thing that it would for traditional chemical; it does not necessarily debunk the EDC low-dose effect argument. Instead, it points to the importance of controlling background exposure to the extent possible in order to keep EDC toxicological results reliable and precise. Studies that do so rigorously may be more reliable than those without such capabilities. The accuracy of different methods was compared in meta-analyses of various in vitro bioassays designed to detect estrogenic activity in environmental waters [205,212]. Studies such as these inform project managers as to which tests are likely to yield results best suited for their research (e.g., most robust and well-aligned with chemical analysis). A combined research approach could help improve accuracy in the face of variation and process contamination [205,213].

2.3.8. Threshold

Traditional RA was designed to analyze only two kinds of chemicals: those with linear-non-threshold responses for cancer-related effects, and those with threshold responses for non-cancer effects

[140]. Advances in MOA research helped evolve these two categories: linear dose–response curve extrapolation if the MOA is DNA-reactive, threshold extrapolation if not DNA-reactive. There is evidence that EDCs comprise a group of chemicals that do not fit into either category as they do not display a threshold for doses linked to all detrimental effects, nor do they always have critical effects or endpoints involving DNA effects. Imposing the concept of a safe threshold on many EDCs is ill-advised for several reasons.

First, some EDCs have been found hormonally active at nano doses [50,146] which means that any "safe" threshold would have to be minuscule, possibly past the point of practicality. Second, for some endpoints there may not be safe (or "no adverse effect level") thresholds at all. For example, thresholds cannot exist for genotoxic EDCs which have an effect at any concentration. The concept of a threshold is also ill-suited where there are effective exposures to substances that are naturally active in an organism, as with endogenous estrogens and androgens [214]. In these cases external EDCs interact with an existing dynamic system of endogenous hormones— substances that have similar effect profiles. Some feel that EDCs can add to the internal load with no threshold [74], while others believe that thresholds are still "the rule" and some cases in which they act via the same mechanism may be the non-threshold exceptions [215]. This interaction between xenoestrogens and natural hormonally active chemicals has been minimally investigated [145,214], and merits further research. Third, chronic exposure to environmental mixtures and combination effects render short term study results from individual chemicals immaterial. Any "safe" thresholds calculated from these will be too limited in scope [181].

To paint a more accurate picture of EDC thresholds (or lack thereof), more information is essential. More pharmacokinetic and pharmacodynamic data are needed to reduce uncertainty and, when available, chemical specific adjustment factors should replace default uncertainty factors to enhance predictive models [216,217]. More information on individual chemicals and common chemical mixtures will improve extrapolations from models and studies to humans. When these data gaps are filled, safe and comprehensive human exposure thresholds can be established, or set at zero.

2.4. Risk characterization

2.4.1. Relative risk

The traditional RA paradigm is meant to standardize the risk accounting process and normalize results so that different risks can be compared. To accurately interpret RA results for EDCs, one must understand the limitations imposed by the extent of uncertainty and the gaps in knowledge affecting the assessment [60]. A risk calculation riddled with potential inaccuracies will not stand on its own as a reliable indicator of absolute risk, nor inform the public whether a given exposure is "safe" or "not safe." However if the unknowns are consistent, the RA will be useful as a point of comparison. In other words: when high uncertainty is involved in an RA but it is repeatable and replicable, the resulting risk values can serve as a basis of comparison rather than offering a final say regarding safety for human health.

In such a case, it might behoove the assessor to treat risk value(s) as comparative tools to assess relative risk as opposed to attempting to calculate absolute risk. For example, values of an EDC RA can help illuminate trends, such as whether risk in an area seems to be growing over time, whether it varies in different locations, whether it is affected by different levels of treatment, or how it compares to estimated risk in other countries. A risk value generated by RA can be compared to risk posed by other monitored stressors [218], and Margin of Safety (MOS) can be used to compare exposure to a particular dose such as that specified in a regulatory standard (e.g., Maximum allowable exposure/Estimated daily intake) [219].

2.4.2. Risk in context

When any form of risk is calculated for EDCs, it should be compared to the risk from EDCs in other sources, and to what is considered tolerable by society. For example, the EDC concentrations found in drinking water are minimal compared to those in food or pharmaceuticals [162]. If the risk posed by EDC exposure discovered in one source pales in comparison to the risk from another source that is known and widely accepted, then the first source should not be the priority for removal unless mitigation efforts involve only trivial expenses. In subsequent (risk management) steps, these considerations will guide researchers and sway decision makers.

Risk should also be gauged in terms of present capabilities for detecting the exposure since analytical technology has made detection of increasingly smaller units of contamination possible [49,220]. This trend implies that some contaminants we are unable to detect currently will be of emerging concern in the near future, and contaminants that can be removed to undetectable levels today (from water, for example) will be detectable tomorrow [162,202,221,222]. Following this logic, if health risk thresholds are set so that no amount of EDC exposure is acceptable, the ability to detect these chemicals at increasingly lower levels will lead to growing financial and energy costs for removal, possibly to the point of diminishing returns and inefficiency [162]. The scientific and risk assessment community will have to grapple with this relationship between detection and public perception of toxicity and risk.

As it stands, most analytical approaches for emerging contaminants are not calibrated based on toxicological relevance, but rather on pushing the limits of method sensitivity [90]. Acknowledging that there are toxicological thresholds below which health is not threatened can frame EDC detection in a way that helps the public judge risk, and helps decision makers react appropriately [212]. Risk assessment methodology for EDCs should be updated based on a more complete knowledge of appropriate thresholds, necessary adjustment factors, and accurate extrapolations in order to clearly demonstrate that detection does not always imply risk.

2.4.3. Appropriate risk communication

It is critical to provide the public with a toxicological context to enrich perception of EDCs so that oversimplification does not lead to extreme reactions driven by fear and ignorance [223], or alternatively to complacency. Concern and action regarding EDCs should be informed by a combination of relevant factors related to exposure (e.g., degree, duration and timing), effects (e.g., whether impacts on the endocrine system are adverse, whether they apply to humans, and whether a link between these can be made by MOA), and socio-political circumstance (e.g., publicly acceptable levels of exposure, detection vs. risk, a government's protection goals).

Proper risk communication addresses unknowns and uncertainty—both quantitative and qualitative—and transparently explains the choices made in a given assessment [224–227]. All information that goes beyond announcing an isolated risk value is important to encourage sufficient precaution, as well as to avoid alarming the public or initiating a policy response with unnecessary or disproportionate expenses [228].

3. Discussion— addressing the challenges

3.1. Summary of research needs

At the moment there is no widely accepted RA paradigm for EDCs [54], however, there are efforts underway to design one that embraces the ways in which these compounds are unique (including temporal and combination factors, and subsequent lack of a

no-effect threshold) [72]. Some basic requirements must be met before the risks EDCs pose to human health (if any) are fully understood. To begin, the definition of EDCs must be finalized, providing a foundation on which to standardize research efforts across the globe. Other key concepts (e.g., adversity) must also be delineated to establish a clear scientific basis from which to determine those effects that are adverse from the perspective of human health [202].

Next, the hundreds of chemicals eligible for priority screening for endocrine disruption should be tested. Further testing for confirmed EDCs can then be prioritized based on potency and potential regional prevalence. To run risk assessments in areas where high EDC concentrations are discovered, appropriate health endpoints for humans would be helpful, based on more complete toxicological data in a shared, centralized, accessible international database [56,72,78,202]. A means of categorizing or ranking EDCs by their relevance to human health should be developed and applied to the database [202]. Studies added to the database should include long-term multigenerational spans incorporating a wide range of doses and signaling pathways [140]. To encourage such efforts, the EPA Office of Research and Development's Chemical Safety for Sustainability Program has called for several advancements [229] which are listed in Fig. 2.

In addition, more pharmacokinetic and pharmacodynamic data are needed to reduce uncertainty, and chemical specific adjustment factors need to replace default uncertainty factors to enhance predictive models [216,217]. The more that is known about each chemical, the easier it will be to ensure accurate extrapolations from cellular and animal level tests to human. For each compound categorized as an EDC, it would be helpful for the areas listed in Table 3 to be investigated thoroughly.

When these data gaps are filled, safe and comprehensive human exposure thresholds can be investigated and finally determined non-existent, or quantified for safety. Methodology for RA of EDCs can be updated based on a more complete knowledge of necessary adjustments, showing that EDC detection does not always mean risk.

3.2. Weight-of-evidence

Weight-of-evidence (WoE) analyses are utilized to compare the evidence, information and/or studies supporting either side of an argument in situations where there is uncertainty [230]. WoE approaches "generally refer to weighing all available evidence, both positive and negative, including human epidemiology data, field data, animal experimental (eco) toxicology studies, in vitro data, (Q)SAR, analogue and category approaches in order to reach a conclusion" [56]. They do not require consensus between all the studies considered, which is one reason that risk assessment can be seen as a subjective scientific process [231]. There are many WoE frameworks to choose from depending on the data under consideration. General WoE best practices are available [232], as well as an EPA Endocrine Disruptor Screen Program WoE guidance document [116,233]. Critical questions for a WoE approach to RA were laid out by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) [202], and are listed in Fig. 3.

In evaluating EDC data, WoE approaches should be applied whenever possible, for example in determining which effects are related to a chemical, the shape of the dose–response curve, and ultimately whether a chemical is an EDC. While there are no standardized WoE approaches for EDCs [64], "Principles of Endocrinology" can be used for a WoE analysis [234]:

- Is the experimental system capable of responding to hormones?
- Is the experimental system responsive to low doses?
- Is the experimental system free from contamination (of natural hormones, EDCs, etc.)?
- Have the results been demonstrated in different laboratories?

These principles can be enhanced further using the modified Hill Criteria to help determine causality [31].

3.3. Interdisciplinary expertise

There are strategies to approach risk assessment which maximize available information, and minimize errors and unknowns. Before undertaking an assessment, risk assessors should consult myriad experts to help interpret the data assembled for a given compound [201]. Relevant fields may include toxicology, biology, biomedicine and chemistry [235], and additional experts in anatomy, physiology, pharmacology, endocrinology, or epidemiology may also be needed depending on the EDC under assessment and how much information on the chemical is already available. When a risk assessment is completed, criteria for reasonableness should be consulted [224]. Finally, science intersects with policy when decision makers help determine how to react to risk findings [236]. Any expert judgments should be described and explained transparently [224,237].

3.4. Toxicology tools

The technology available for studying EDCs has evolved beyond the mere detection of chemicals and determination of their amounts. Traditionally, to test whether a medium such as air or water met regulatory standards regarding toxicity, chemicals would be tested for one by one and their concentrations calculated. The results of such monitoring schemes are typically limited to concentrations, and only for the specific contaminants being targeted for analysis [238]. The need to understand chemical effects and potency has driven the development of biosensors.

Present understanding of interactions in most chemical combinations is still inadequate to reliably predict biological responses [178], but multi-chemical tests that directly measure biological responses are improving [178,213] and may eventually reduce the need for conventional single-chemical monitoring. Testing for bioactivity places increasing emphasis on measuring the effects as opposed to the amount of a contaminant [49]. This allows regulating agencies such as the EPA to capture the extent of a biological effect of interest, regardless of the chemical(s) inducing it, and determine whether there is risk to human or ecological health. This is based on one or more endpoints of concern, in an integrated analysis of the medium as opposed to being based on a subset of the chemicals in a medium. In this way, the step connecting the amount of a chemical measured and the estimated effect of that chemical can be omitted, and mixture effects accounted for without guesswork. This reduces uncertainty on both fronts, and will increasingly strengthen regulation [178].

Typically bioactivity tests will involve high- and medium-throughput assays. Application of these methods using existing bioassays is already being piloted in some US states such as California [178]. Some bioassays measure responses in populations of living organism, and are advantageous in that they demonstrate in vivo responses to EDC exposure [146]. Aquatic animals tend to be particularly sensitive to these compounds so amphibians and fish are common choices for these assays, as well as insects and birds. Some fish have even been genetically altered to enhance their reaction or to manifest it in ways that are easy to measure (e.g., florescence protein expression) [146].

Bioassays can also be cellular, such as the E-Screen which takes advantage of the fact that EDCs interact with estrogen receptors and elicit biological responses similar to those of natural steroid

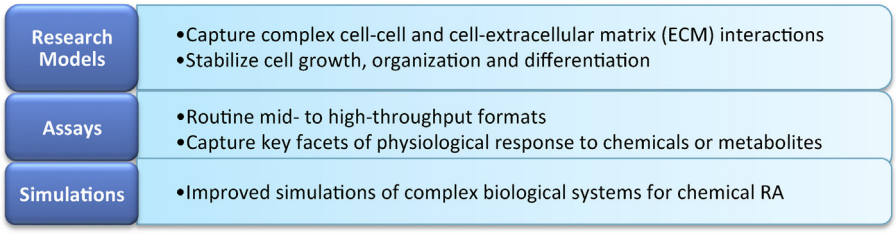

Fig. 2. EPA's EDC recommended areas for advancement.

Table 3
EDC Research Needs and Priorities.

	Research Needs
Fundamental EDC Information Gaps	Mechanisms and modes of action Sources and paths through environment Response thresholds Combination effects Low dose effects Dose–response (potentially non-monotonic) curves in multiple species, ending with humans
Priorities of International Workshop on EDC RA Methodology [199]	Characterization of range of human exposure/levels in body fluids/tissues Determination of critical windows for exposure relevant to man Identification of vulnerable members/groups of population Understanding of impact of exposure to multiple stressors Examination of effects over much greater dose range
Endocrine Society Recommended Projects to Inform Policy on Human EDC Exposure [28]	Large prospective studies to examine relationship between exposure (particularly to agents with estrogenic and antiandrogenic activity), and relevant endpoints identified in Society's report. Identification of populations or subgroups with high exposure to EDCs, and exposure-response analysis among them More epidemiological studies that incorporate measurement of exposure to multiple EDCs (for health effects of mixtures and biomarkers of EDC exposures), and relevant outcomes incorporated Observations from occupational/environmental exposures in humans and corresponding disease states to chose animal studies and EDC targets Molecular studies in vitro and in vivo to identify pathways for EDC influence of endocrine tissues Expansion of studies of mechanisms by which EDCs affect neuroendocrine systems (and other endocrine related systems) Improved differentiation/characterization of roles of steroid and non-steroid pathways, and more information on low dose effects of EDCs Studies across different endocrine and reproductive system on transgenerational, epigenetic effects of EDCs, and interactions of EDCs with central nervous system developmental processes dependent on thyroid hormones or sex steroids Studies on the effects in animal stem cells or progenitor cells in different tissues, possibly used as reporters in early biomarking, to help decipher EDC-sensitive genes

hormones [23,239]. The E-Screen is an integrative intercellular bioassay that uses MCF-7 human breast cancer cells which proliferate when put into contact with chemicals that directly or indirectly activate the estrogen receptor [23,185]. This highly sensitive test can be used to detect estrogenicity, and it can thus act as a screen to indicate which substances or compounds merit further testing and help avoid the release of EDCs that could have an adverse effect on exposed organisms. This screening application extends to other cellular bioassays including YES using a colometric response, ER-CALUX using a luminescent response, E. coli using a luminescent response, HEK 293 cell using a human embryonic kidney response, and an infrared bio-amplification using a mammalian cell [146] (see Appendix C for table summarizing cellular bioassays).

Alternatively, non-cellular assays are similar in practise to cellular assays, but do not require a whole cell and thus avoid complications caused by membrane permeability or other elements of cell functioning [146]. These include the enzyme-linked immunosorbent assay (ELISA) and the enzyme-linked receptor assay (ELRA) in which estrogenic EDC responses are quantified using laboratory equipment. Biosensors, electrochemical sensors, fluorescent indicators, and microarray relative binding assays are also helping improve EDC monitoring capabilities [146].

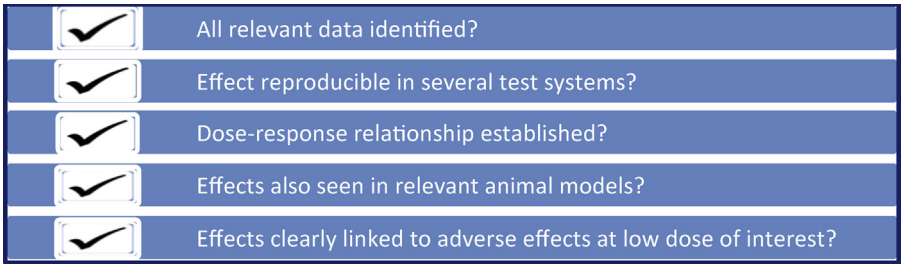

Fig. 3. WoE approach to risk assessment.

Cellular and non-cellular assays offer a standard by which to define estrogen activity [240], and are arguably more ethical than tests using a whole animal [23]. By measuring the total endocrine effect of environmental contaminations in a given pathway, bioassays may more comprehensively assess exposure than chemically measuring levels of each known xenoestrogen. This is because there may be unidentified EDCs in the mix, and because multiple EDCs can display unknown combination effects. They thus make useful screens.

Still, such bioassays present several problems. First, calculations based on cell proliferation tests may not properly account for the actual pharmacokinetics and pharmacodynamics of chemicals, thus oversimplifying a complicated process and providing an incomplete picture. In vitro testing can result in false positives [241] as they detect for mechanistic relationships and are not always highly discriminatory. Second, the exclusive use of in vitro tests to screen for estrogenicity in biological testing may underestimate estrogenicity in certain sources because they miss the presence of non-estrogen compounds that produce estrogenic effects through indirect mechanisms [242]. Finally, due to the tremendous variety in EDC endpoints and modes (and mechanisms) of action, a single bioassay is not sufficient to test the effects of potential endocrine disruption on a whole organism.

To help safeguard against such problems, screening tests should include more physiological and toxicological end points from whole animal studies designed to offer a representative relation to human physiology [30]. In theory, a suite of bioassays is needed to provide both the mechanistic and apical information required to characterize an EDC [57], but more research is required to develop the appropriate combinations. To ensure comprehensiveness, a combination of in vivo and in vitro tests may be needed. Chemical and biological tests can be run in tandem to compare and enhance results [243] using potency factors such as estradiol equivalents (EEQ) [179]. Chemical tests using gas chromatography mass spectrometry (GC–MS) [244], liquid chromatography mass spectrometry (LC–MS), and tandem mass spectrometry have proven effective [182,213,245,246]. The sum of individual target compounds from chemical testing expressed in EEQ concentrations can be compared to the total biological response in the E-screen [179].

There has also been a push to design and implement integrated testing strategies to replace more iterative processes that involves a default animal test, then a cell-culture, then computer based methods to define MOA, and finally interpretation and further balancing of results [163]. Today, the approach used depends on what is needed and what is known— first using all existing information about a substance and similarly structured substances without animal testing, then targeted animal testing used only when necessary. This approach ideally begins with a screening process to identify suspicious chemicals and moves on to a more sophisticated confirmation step. Having an intermediate phase can help minimize false positives, and allows for endpoint analysis to determine which assessments tend to lead to classification as a toxin [163]. The EPA has revised its toxicity testing strategy to this end with its ToxCast program [247]. Also, in the EU an integrated strategy from the REACH guidelines for industry uses animal testing only as a last resort, calling for more flexibility and customized approaches [163]. In some regions, limited resources, equipment and expertise may hinder implementation of new technologies, but information-sharing and transparency can help bridge these gaps.

Any future integrated testing strategy may need to be built from scratch to best accommodate the many new techniques in toxicology such as the growing application of in vitro testing that can utilize human stem cells [163]. Contributions from bioinformatics and biotechnology can also help move the field forward [248,249]. Three technologies are especially valuable and were only introduced recently: advances in “omics” (e.g., genomics and proteomics), imaging techniques, and automated testing platforms. In combination these facilitate higher throughput to run more tests faster, and they make it easier to mine for patterns that characterize certain toxic effects and integrate them with other areas of science [163]. To move these efforts forward, more information on full “adverse outcome pathways” (discussed in the Section 3.5 below) is needed, as well as associations between these and apical outcomes. Based on these modifications, bioactivity thresholds can be developed similar to the reference doses of today, but applicable to a specific spectrum of bioactivity.

Although developing such a new chemical testing strategy and vetting it internationally will not be simple [58], this will streamline the testing process and address many of the limitations facing toxicology and epidemiology today. It can ultimately help integrate the exposure and dose–response assessment steps of risk assessment.

3.5. Status of RA for EDCs

Due to the uncertainties, unknowns and complexities, there is an argument claiming that traditional toxicological RA processes cannot be used to reliably determine the health effects of EDCs. Some argue that attempts to stretch data using models just introduce more assumptions and potential for error. Others express a concern over applying traditional RA to EDCs prematurely, before the difficulties are overcome. They feel that inaccurate risk values could lead to a dangerous sense of false comfort and acquiescence of hazardous situations in the field, and subsequently to unnecessary exposure. Their conclusion regarding the level of uncertainty in an RA of EDCs is that the consequences of being “wrong” are too great to justify any potential benefits [250,251].

The opposing side of this argument focuses on the fact that human exposure to EDCs is well documented, so while calculating an absolute quantitative risk value may not be possible considering the uncertainties, EDCs can and should undergo some form of RA [252,253]. A recent report from the European Food Safety Authority [57] concurs:

“to inform on risk and level of concern for the purpose of risk management decisions it is the opinion of the SC that risk assessment (taking into account hazard and exposure data/predictions) makes best use of available information. EDs can therefore be treated like most other substances of concern for human health and the environment, i.e., be subject to risk assessment and not only to hazard assessment.”

EDC risks to humans potentially increase every day, therefore it is proposed here that RA of EDCs should be undertaken now by leveraging available methods and existing analytical tools. Such application of existing RA methods to EDCs should be undertaken prudently; any risk results must be communicated with the clear warning that results are uncertain at best, inaccurate and misleading at worst. Carried out responsibly, an RA of EDCs can help identify priority areas as a step in exposure reduction.

Ultimately, it would be ideal to have new approach specifically developed for risk assessment of EDCs [62]. New initiatives to enhance RA of EDCs focus on mapping the compounds’ MOAs to help highlight those events most relevant to assessing risk. One such project is called the “Human Toxome Project,” which is building a public database to house all the endocrine disruption pathways of toxicity [83]. A related tool gaining popularity in ecotoxicology and risk assessment is called Adverse Outcome Pathway (AOP), a transparent way to link exposure with adverse effects [254]. “An Adverse Outcome Pathway (AOP) is a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment” [255].

In effect, an AOP is much like an MOA with more detail, and more emphasis on the connections between different levels of organization. An AOP RA incorporates risk-related events from the chemical source all the way up to the last human health endpoints and population level effects, which can help organize and clarify the connections in between. As the framework is strengthened and standardized, it will be able to help incorporate, relate, and extrapolate between different levels of information required for conducting an EDC risk assessment [116,254].

When undertaking RA of EDCs it is important to accommodate the unique characteristics of these chemicals whenever possible. Factors such as vulnerable windows of exposure and potentially NMDR relationships should be addressed in the formal methodology [72,74]. Characterizing uncertainty and explaining all choices explicitly will provide maximal transparency [237,256,257]. This openness can at best help benchmark risk estimates to inform EDC management decisions today, or at minimum can help overcome the pitfalls facing human health risk assessment of EDCs moving forward.

3.6. The future: formal EDC RA protocol components and applications

As detection and screening technology continues to improve, tools such as AOP mature, and data on chemical characteristics including mechanism accumulate in global databases, research priorities surrounding EDCs will be better delineated and the key concepts more clearly defined. Questions and debates will give way to concrete definitions offering a deeper understanding of this highly diverse chemical group. Using a WoE approach that is complimented by diverse expertise, the more robust information about EDCs will make it possible for government agencies and NGOs to develop a systematic RA protocol for these chemicals. These guidelines should leverage current methodology (from existing chemical, environmental and human health RA steps) when it applies, and bring to bear newly available information.

Anticipated components of future RA procedures include more reliance on in vitro and in silico (i.e., performed on computer or with computer simulation) methods, increased use of "omics" based endpoints enabling earlier detection/improved sensitivity, and progressive change to a MOA basis for evaluating hazardous properties [202]. Mapping the interconnectivity of effects and mechanisms of EDCs at different levels will help model the chemicals' often ubiquitous environmental transport and human exposure effects to identify appropriate endpoints of concern. Better exposure measurements and estimates will help identify chemicals of priority, and effective exposure will become easier to gauge with improved extrapolation. Integrated monitoring and testing technology will be developed with an eye to mixture effects instead of individual chemical concentration. Combined research approaches in which a suite of tests is tailored to a given research goal will help improve accuracy, and decrease inter-laboratory variability. These tests will include long-term, robust, multigenerational studies to capture chemical persistence and bioaccumulation.

In the traditional RA paradigm, cancer and non-cancer cases were considered two distinct categories: while a low enough dose of non-cancerous chemical was treated as "safe," any exposure to cancerous materials was considered dangerous. Consequently chemicals in the carcinogenic category that lacked a perceived safe threshold were treated differently in the dose–response assessment step of traditional RA. These two approaches were thought to encompass the entire chemical universe. There is growing recognition that this dichotomy results in oversimplification [31]. Some CECs—such as EDCs—do not fit into either category: they are not necessarily cancerous, but most cannot be assigned any practical safe threshold of exposure. (A list of existing RA methods used for genotoxic/carcinogenic chemicals was published in a 2009 report by the EC [32]). Due to their low dose effects (e.g., xenoestrogens that are hormonally active at sub-ng/L levels) and temporal considerations (e.g., latency and critical windows), any exposure could potentially be harmful. Any prospective EDC RA protocol should account for the fact that while EDCs may not have a safe threshold, they do have identifiable low-dose effects (e.g., NMDRCs) and unique exposure characteristics (e.g., long-term, timing-dependent).

Any type of RA result for EDCs should be communicated alongside abundant toxicological context so that risk can be judged reasonably, increasing the likelihood that public and legislative reaction will be rational and proportionate [223,258]. Where uncertainty is high and risk assessment results are reliable and repeatable, comparative risk tools can generate trends and reports about relative performance. In cases where a chemical is found to pose a pressing danger to human health, it may be necessary to develop and promote as many EDC substitutes or EDC-free materials as possible to mitigate contamination [65]. Both scientists and policy-makers will be called upon to respond and cooperate.

To be politically feasible, a new method for RA must be relevant to the full range of effects on human health, scientifically valid, reproducible, ethically acceptable, of low (or moderate) cost with potential for high throughput, and suitable to assess effects at doses that reflect likely human exposure [202]. To encourage worldwide acceptance, a new RA protocol appropriate for EDCs must be consensus-driven, and able to accommodate new knowledge as it arises. This constitutes a tall order, but meeting these objectives will allow RA for EDCs to be standardized, transparent and feasible for agencies that must make decisions with limited resources. Such a protocol will help protect the public with appropriate regulation in the face of the growing challenge posed by exposures to EDCs.

There are several EDC sources and possible exposure points that could benefit from a RA. For example, recycling wastewater is a relatively young technology that, while helping ameliorate water scarcity problems, may present health hazards which have not yet been fully characterized. Wastewater reuse is becoming an increasingly attractive option in regions where water shortages are exacerbated by growing populations and climate change. However, the types of sewage treatment used historically may not remove all micro-contaminants including EDCs [8,259,260]. As synthetic compounds from human use add to EDCs that occur naturally, concentrations rise in water sources that serve as sinks for pollutants in the environment [239]. Already many countries have detected high levels of EDCs in their water supplies [87,222,261], and EDCs are emerging as a challenge to wastewater treatment [4,192,262,263] that guidelines for a customized EDC RA could help address.

Another EDC exposure scenario with global implications is prevalence in food such as soy products [147,264–267], aquatic food sources including fish, flavoring substances [246], and leaching into food by "contact materials" which include plastic packaging, laminate coatings, and plastic bottles [268,269]. Some countries have responded with warnings and regulation specific to high-risk groups (e.g., infants and women with breast cancer) [265], and precautionary responses such as those by Canada [270] and the EU [271]. The EU has imposed restriction on use and sale of certain EDCs in foods, plastic products, and certain migration limits [272].

The US has put a few regulations in place to protect vulnerable populations [25], but has a generally more conservative approach to regulating EDCs. This is in part the result of a lively debate between environmental organizations and consumers demanding clear regulation [273], versus stakeholders such as chemical companies and federal health and risk organizations that argue against premature banning of certain EDCs (e.g., phthalates) for which they believe the benefits outweigh the risks [71,274–276]. The lack of national level

EDC laws in the US will lead to varying exposures across states and risk levels across the nation.

Resources invested into improving and applying EDC RA protocol could provide countries such as the US with a more objective basis for influencing EDC policy. Risk assessment could help reducing the role of lobbying and increasing the role of science in reacting to potential threats from EDCs; on the one hand culling potentially misguided alarmism and spending, on the other hand protecting public health from this idiosyncratic group of chemicals.

4. Conclusion

EDCs are globally ubiquitous, and have been confirmed harmful to wildlife. Human exposure to environmental EDCs is inevitable, most often to mixtures in chronic low doses which add to the concentration of natural endogenous hormones. It is difficult for regulators to know which EDCs pose danger to human health severe enough to justify a reaction, or even to justify analysis comparing potential interventions. Risk assessment is an invaluable tool offering the opportunity to systematically assess the potential risk posed by a class of chemicals that is not yet fully understood. The unknowns and uncertainties render an informative RA of EDCs difficult but not impossible. Sharing of toxicological, epidemiological, mechanistic and other EDC information in cooperative international databases can help improve provisional RAs. While it may be too soon to calculate absolute risk values, comparative RAs can leverage current methodology to garner insight— albeit coarsely— while an improved protocol is tailored specifically for EDCs. Alongside abundant communication explaining the limitations of initial results, such work can help regulators to begin ranking EDCs by potency and prevalence, maximizing limited resources and helping to carve out a rational path forward.

Disclaimers

The authors claim no conflicts of interest. The MERC grant funding the larger research project behind this article came with no stipulations in regards to the preparation of this work or the decision to publish.

Acknowledgements

This work was conducted under a joint research initiative between Ben-Gurion University of the Negev and The Water and Environmental Development Organization (WEDO), and supported by the USAID Middle East Regional Cooperation (MERC) Program (grant TA-MOU-11-M31-015). The authors thank Professor John Evans (Harvard School of Public Health) for his valuable ideas regarding early drafts. We also thank Dr. Shane Snyder (University of Arizona) for his advice on risk assessment and EDC analysis. We thank Dr. Lynne Haber from Toxicology Excellence for Risk Assessment (TERA) for her insights into dose–response issues, and her thoughtful draft review. Finally, we thank two reviewers for their thorough editing and helpful suggestions.

Appendix A.

The following challenges that EDCs pose to traditional risk assessment are not unique to this chemical group, but have a pronounced enough effect to merit discussion.

S1 Ubiquity and transport

The extent of an individual's exposure to a chemical depends on multiple factors such as age, habits, ingestion rates, inhalation rates, location, concentration in a given media, and so on. As synthetic and natural EDCs have proven globally ubiquitous [87,88] in the environment and in the human body [89], exposure to EDCs is highly variable and difficult to calculate in large populations [30]. Compounds such as polybrominated diphenylethers (PBDEs, used as flame retardants) and polychlorinated biphynls (PCBs, used as lubricants) have been identified thousands of miles from their sources [177] in regions where they were never used such as the Arctic [277–280]. EDCs are especially prominent in water and aquatic environments [50], but they travel in all environmental mediums: air [281–283], water [121], sediments [284,285] and live tissue [275].

Different countries and organizations have developed various models to assess chemical transport, including the European Union System for the Evaluation of Substances (EUSES), the Canadian Environmental Modelling Centre (CEMC) models, and CalTOX created by California and used by the EPA as well as countries in Asia and Europe [286]. Transport models for EDCs are complex and, though useful for stretching observations into predictions, they can also be affected by the underlying uncertainty surrounding EDCs. These models have all been studied independently, and uncertainty within the models was found to be higher than uncertainty between them [287]. They have all been deemed sufficiently accurate for specific domains of chemical properties. Accordingly, minimizing inaccuracies simply means selecting the appropriate model for a given question and context [286]. Such models can all contribute to a better understanding of transport mechanisms in the environment for soil, water and air [146] which will help measure human exposure to EDCs.

S2 Calculating exposure

Even when an exposure scenario is well understood and actual (external) exposure calculated based on the concentration of an EDC received by an organism in a medium such as air or food, the "effective exposure"— the quantity of chemical actually reaching the target organ or receptor [288]— might not be obvious. Simply put: External Dose = Internal Concentration × [Clearance/Absorbed fraction] [289]. To account for variations that affect the compound concentrations at the target site [177] by relating external exposure to internal exposure, adjustments can be made using standard uncertainty factors, or better yet with personalized biomonitoring data. This can help account for species or individual differences [290] including ingestion rates, inhalation rates, dermal absorption rates and perhaps the less obvious pharmacokinetic [291] and pharmacodynamic factors.[1]

Pharmacokinetics refers to what the body does to compounds: the time course of chemical absorption, internal distribution, metabolism, and excretion which determine the compound's concentration and rate of availability to the relevant receptor site. Pharmacodynamics refers to what the compound does to the body: the relationship between chemical concentration at the cellular level, and the nature, time course and intensity of effects, adverse or benign. It determines the efficacy of the chemical's activity at the site of action and applies to both the parent molecule and its metabolites. It can be difficult to quantify this sequence of events, so measuring equilibrium bioconcentrations is sometimes used a surrogate parameter to better understand observed effects [292,293].

Dose–response assessment and exposure assessment are closely connected in RA. If dose–response value is calculated based only upon the external dose without explicit corrections for inter-

[1] These are sometimes referred to as toxicokinetic and toxicodynamic factors when dealing with doses outside of therapeutic range, but the prefixes toxico- and pharmaco- are considered synonymous for the purpose of this paper.

nal processes, the RA will not as closely reflect reality. To encourage RA accuracy, necessary adjustments can be made using one or more mathematical dosimetry models, adjustment factors, and physiologically based pharmacokinetic or pharmacodynamic models [48].

S3 Study limitations

A mix of environmental, indoor, and transgenerational exposure potentially hampers the ability of assessors to isolate and quantify risk to human health from EDCs. To help address gaps in data, epidemiologists and toxicologists conduct studies to assess exposure to EDCs as well as the cascade of consequences from that exposure. These scientists must weigh accuracy against resource requirements when designing such a study. In epidemiological studies, indirect measurements are often relied upon which derive exposure estimates from existing data [294]. This is because direct measurement for a full exposure scenario of an EDC RA of human health is extremely costly in both time and money. However, indicator measurements, diaries, questionnaires, models of exposure or probabilistic exposure [295] or other such proxies introduce additional uncertainties into a RA. For examples, when surveys are used to gauge exposure in epidemiology, issues regarding inaccuracies from recall bias or under-representative samples are introduced. Also selected population samples might not be representative simply by chance [19].

Direct exposure monitoring programs for a human target of interest involving measurements taken at the "point of contact at the moment it occurs" [294] are better, but require very high investments in time and money. The result is an imbalance regarding available exposure data across different EDCs. For some compounds there is only minimal information, while for others, numerous estimates of exposure exist based on intake through known routes or measurements of aggregate exposure from biomonitoring [89]. Programs such as the EPA's ExpoCast can help translate computational toxicology findings into useful approaches and metrics for evaluating chemicals in relation to their potential for biologically relevant human exposure [296,297].

S4 Extrapolation

Epidemiological data provides the most realistic and wholistic cause-effect information, but they are not available for all chemicals and certainly not for all mixtures. Due to barriers to direct human testing and other study limitations, sometimes toxicological results from one kind of dose–response study are extrapolated to a scenario of interest. Extrapolation is the process of estimating, beyond the original observation, the value of a variable on the basis of its relationship with another variable [298]. In theory, extrapolation can be used to apply results from tests on one species to a different species (i.e., interspecies extrapolation), between levels of organization in an organism (i.e., cell-to-human or tissue-to-whole-organism extrapolation), between dose size or between exposure duration (i.e., sub-chronic to chronic) [151].

Extrapolation generally relies on calculations that cannot account for all possible scenarios and thus are not completely accurate. For instance, doses tested in toxicology at best approximate a real situation. As a result, different degrees of uncertainty are compounded every time results for one level of organization or species are extrapolated to another [16,19,299]. To illustrate, interspecies and cell-to-human extrapolations introduce uncertainty due to physiological and pharmacokinetic differences that are not accurately or fully captured in the estimated transfer [19,300–302]. In another scenario, results from rodent studies on endocrine effects can be extrapolated to humans in theory, but must be tested in practice [303]. For example, an EDC mode of action determined in rodents can result in different outcomes for humans (e.g., hormonally-induced thyroid tumors) [304]. This is why tests on animals can help prioritize chemicals for further testing, but do not indicate human hazard with finality [305,306].

To compare the biological significance of human EDC exposure with that of an animal, the species dependence of sensitivities in animal models must be considered, as well as a toxic endpoint's relevance to human health [60]. That said, there are relatively few well-documented cases in which endocrine effects on animals have proven to be non-relevant to humans [307], so extrapolations are the best option in the face of ethical barriers to human testing. However, despite its usefulness, extrapolating human dose–response information is problematic. The (usually high) doses given to animals are often not representative of the doses typically metabolized by humans [19,163]. To help temper this problem, there are a variety of methods that can be used for interspecies scaling to calculate the human equivalent dose (HED) of a compound [308], including body weight scaling, surface area scaling and pharmacokinetic modeling.

In addition, measures are usually taken for a single chemical at dosages that tend to be higher than environmental exposure levels [30]. This is not always the most appropriate information for a RA because response patterns might be unique in high doses due to intricate physiological and/or pharmacokinetic processes. This would render low dose extrapolation inappropriate [30,56]. Uncertainty mounts when extrapolating results of a high dose–response test to a low dose test for EDCs that display a non-monotonic dose–response curve and unexpected low dose responses [56]. Duration of exposure calculations come with additional uncertainty factors [151,309], and can be further complicated by long latency periods and transgenerational characteristics of health problems induced by EDCs [60]. These areas of ambiguity all threaten the quality of the data by complicating the verification of effects [118,137].

A greater understanding of EDC modes and mechanisms of action in each species, at each level of organization, and between various doses and time-frames will help improve the accuracy and reduce the uncertainty of extrapolations. There is a general lack of data in this area [163] that can be alleviated by studies on how one species models for another, and establishing chemical-specific adjustment factors to replace extrapolation with accompanying default uncertainty values [151,235]. Any studies investigating these relationships should be added to a global database of EDC information in order to locate gaps in data and reduce redundant research.

In addition to these incremental increases in knowledge, larger scale mapping of known information through frameworks such as Adverse Outcome Pathways [255] and chemical activity prediction through Quantitative Structure Activity Relationship (QSAR) modeling [151,310] can help illuminate broader patterns in how EDCs function and integrate testing strategies. When models are used, results can be extrapolated to actual human exposures, for example by employing probability trees (weighted model averaging) which is the process of estimating a value under multiple models and averaging the results according to the likelihood that each model is accurate [311,312]. Alternative methods include three-dimensional modeling of structural responses to EDC exposure [313,314] which could improve accuracy of frequency, severity, and uncertainty.

Appendix B.

Table 4"Endocrine disrupting chemicals (EDCs) can be grouped in multiple ways. In this table known or potential EDCs are grouped into 11 categories with examples of individual EDCs. Bolded chemicals were selected since they are regarded to be of specific interest as EDCs, and are described in more detail in the text" [29].

Classification	Specific Examples of EDCs
Persistent and bioaccumulative halogenated chemicals	
Persistent Organic Pollutants (POPs) (Stockholm Convention) (section 3.1.1.1)	PCDDs/PCDFs, **PCBs**, HCB, **PFOS**, **PBDEs**, PBBs, Chlordane, Mirex, Toxaphene, **DDT**/DDE, Lindane, Endosulfan
Other Persistent and Bioaccumulative Chemicals (section 3.1.1.2)	**HBCDD**, SCCP, PFCAs (e.g. **PFOA**), Octachlorostyrene, PCB methyl sulfones
Less persistent and less bioaccumulative chemicals	
Plasticizers and Other Additives in Materials and Goods (section 3.1.1.3)	Phthalate esters (**DEHP**, BBP, DBP, DiNP), Triphenyl phosphate, Bis(2-ethylhexyl)adipate, n-Butylbenzene, Triclocarban, Butylated hydroxyanisole
Polycyclic Aromatic Chemicals (PACs) including PAHs (section 3.1.1.4)	**Benzo(a)pyrene**, Benzo(a)anthracene, Pyrene, Anthracene
Halogenated Phenolic Chemicals (HPCs) (section 3.1.1.5)	2,4-Dichlorophenol , Pentachlorophenol, Hydroxy-PCBs, Hydroxy-PBDEs, Tetrabromobisphenol A, 2,4,6-Tribromophenol, **Triclosan**
Non-halogenated Phenolic Chemicals (Non-HPCs) (section 3.1.1.5)	**Bisphenol A**, Bisphenol F, Bisphenol S, Nonylphenol, Octylphenol, Resorcinol
Pesticides, pharmaceuticals and personal care product ingredients	
Current-use Pesticides (section 3.1.1.6)	2,4-D, **Atrazine**, Carbaryl, Malathion, Mancozeb, **Vinclozolin**, Procloraz, Procymidone, Chlorpyrifos, Fenitrothion, Linuron
Pharmaceuticals, Growth Promoters, and Personal Care Product Ingredients (section 3.1.1.7)	Endocrine active (e.g. Diethylstilbestrol, Ethinylestradiol, Tamoxifen, **Levonorgestrel**), Selective serotonin reuptake inhibitors (SSRIs; e.g. **Fluoxetine**), Flutamide, 4-Methylbenzylidene camphor, Octyl-methoxycinnamate, Parabens, **Cyclic methyl siloxanes** (D4, **D5**, D6), Galaxolide, 3-Benzylidene camphor
Other chemicals	
Metals and Organometallic Chemicals (section 3.1.1.8)	Arsenic, Cadmium, Lead, Mercury, **Methylmercury** Tributyltin, Triphenyltin
Natural Hormones (section 3.1.1.9)	17β-Estradiol, Estrone, Testosterone
Phytoestrogens (section 3.1.1.9)	Isoflavones (e.g. Genistein, Daidzein), Coumestans (e.g. Coumestrol), Mycotoxins (e.g. Zearalenone), Prenylflavonoids (e.g. 8-prenylnaringenin)

Appendix C.

Examples of single cell bioassays for detection of e-EDCs (estrogenic EDCs)

Table 5 Sample EDC bioassays [146].

Common name	Cell type	e-EDC effect	Reference
E-SCREEN	MCF-7 breast cancer cells	Cell proliferation response	Soto et al. (1995)
Yeast Estrogen Screen (YES) – *including LYES and BLYES variations as well*	Various (*Saccharomyces* spp., *Cryptococcus* spp., and *Candida* spp.)	Colometric & luminescent response	Arnold et al. (1996b), Routledge and Sumpter (1996), Fang et al. (2000), Silva et al. (2002), Legler et al. (2002b), Schultis and Metzger (2004); Sanseverino et al. (2005)]
ER-luciferase assay with HEK 293 cells	Human embryonic kidney (HEC)	Luminescent response	Pawlowski et al. (2003)
NA	*E. coli*	Luminescent response	Gu et al. (1999)
Estrogen responsive chemically activated luciferase expression (ER-CALUX®)	T47D human breast adenocarcinoma cell	Luminescent response	Legler et al. (2002b); BioDetection Systems, Amsterdam, The Netherlands[a]
IR-bio-amplification	Mammalian cells	Cellular function	Holman et al. (2000, 2003)

[a] Commercially available product.

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