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ORIGINAL ARTICLE

Firm human evidence on harms of endocrine-disrupting chemicals was unlikely to be obtainable for methodological reasons

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Abstract

Background and Objective: Despite plausible laboratory evidence about harms of endocrine-disrupting chemicals (EDCs), systematic reviews and meta-analyses of epidemiological studies do not consistently find such harms. The purpose of this article was to discuss why solid human evidence on EDCs was unlikely to be obtainable even though they are truely harmful to humans.

Results: Indeed, there are insurmountable methodological limitations in human studies. They include unpredictable net effects of diverse EDC mixtures, low reliability of exposure assessment, nonmonotonic dose-response relationships, nonexistence of an unexposed group, and complicated interactions with diet and obesity. The exposome is never free from these methodological issues. Therefore, the most persuasive evidence about human harms of EDCs may be increasing EDC-linked diseases at population levels, but traditional epidemiological studies of EDCs, especially short-lived and widely used EDCs, fail to provide consistent results. Nevertheless, human studies of EDCs with long half-lives, for example, persistent organic pollutants (POPs), are still worthwhile because there are fewer methodological issues. Also, they can play a role as surrogate markers of lipophilic chemical mixtures. Notably, although POPs are well-known EDCs, human findings on POPs cannot be attributed to common hormone-disrupting properties because the net effect of diverse EDC mixtures cannot be reliably predicted even with POPs. Homeostasis disruption through mitochondrial dysfunction may be more relevant to such effects.

Conclusion: Logical inference should play a primary role in judging harms of EDCs in humans.

Keywords: Chemical mixtures; EDCs; Epidemiology; Nonmonotonic dose-response relationship; Reliability

1. Introduction

Researches about endocrine-disrupting chemicals (EDCs), any compounds that interfere with endogenous endocrine action, have dramatically increased since the late 20th century. In official reports issued by the Endocrine Society [1,2] and the World Health Organization (WHO)/United Nations Environment Program (UNEP) [3], EDCs are related to many health problems, including reproductive disorders, hormone-sensitive cancers, neurodevelopmental impairment, thyroid dysfunction, obesity, and diabetes [1−3].

However, there is controversy about whether or not EDCs cause adverse outcomes in humans. After the publication of the 2012 WHO/UNEP report, there was rebuttal correspondence between researchers who criticized the report [4−7] and those who supported the conclusion [8−12]. Lack of solid human evidence is a common criticism from opponents of the WHO/UNEP report. Many critiques of dissenting researchers could be distorted because of their links to chemical industry [12], but the issue on human evidence deserves further scientific discussion because inconsistent results from recent systemic reviews and meta-analyses of epidemiological studies about common EDCs such as bisphenol A (BPA) or phthalate seem to support the opponents' position [13−16]. If this inconsistency is considered as lack of human evidence, huge economic costs of disease burden related to the exposure to EDCs estimated in the EU and USA [17,18] can be sequentially

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What is new?

Key findings

- Human studies on endocrine-disrupting chemicals (EDCs) suffer from probably insurmountable methodological limitations.
- The exposome is never free from these methodological issues.

What this adds to what was known?

- The most persuasive evidence about human harms of EDCs may be increasing EDC-linked diseases at population levels, but traditional epidemiological studies of EDCs, especially short-lived and widely used EDCs, fail to provide consistent results.
- Human studies of EDCs with long half-lives are still worthwhile because of fewer methodological issues.

What is the implication and what should change now?

- Human effects of EDCs should be inferred by the philosophy of evidence integration.

problematic as some researchers criticized [19,20] because this estimation is only reasonable under the assumption of causality in humans.

This article was written to discuss why we cannot obtain solid human evidence on EDCs even though EDCs are truly harmful to humans. Researchers tend to have the optimistic opinion that limitations will be overcome with advanced technology and meticulous study design. However, our perspective is that many are innately insurmountable limitations. Based on the discussion below, we will claim that human effects of EDCs should not be determined based on findings from epidemiological studies, but rather that the philosophy of evidence integration should be used. Also, we will suggest what kinds of human studies of EDCs remain useful despite all these impediments.

2. Methodological issues in human studies of EDCs

2.1. Issue 1. Mixtures

Humans live with simultaneous daily exposure to a tremendous number of diverse EDCs, but most research on EDCs, laboratory or epidemiological, has been performed with individual compounds. Although there are a limited number of laboratory studies about EDC mixtures, they mainly investigate several similarly acting compounds. For example, the strong synergic effects of several weak estrogenic compounds have drawn a lot of attention [21,22]. Mixtures of antiandrogens or thyroid-disrupting chemicals also reported additive or synergic interactions [23]. Thus, researchers tend to assume that effects of different classes of EDCs may be additive or even synergistic, as was assumed in the first official report from the Endocrine Society [1].

However, combination effects of EDCs with different endocrine-disrupting properties have demonstrated modulating or antagonistic interactions that cannot be predicted by adopting additivity or synergistic concepts [24–26]. Also, even though diverse EDC mixtures were used in experiments, mixtures used in in vitro or in vivo experimental studies have still been very simple, artificial mixtures, compared with real world EDC mixtures. Thus, research on naturally occurring mixtures found in marine and freshwater can provide a more relevant insight into effects of EDC mixtures in humans. For example, when the effect on ovarian follicular cell steroidogenesis was evaluated with three types of mixtures, which varied in content and concentration of individual chemicals (10 to 1000 times per chemical), all three mixtures demonstrated similar endocrine-disrupting effects [27]. Also, they observed nonlinearity within the range of background concentration, higher response in low-dose ranges, compared with high-dose ranges [27], which will be further discussed below.

Chemicals currently classified as EDCs are those that act as direct agonists or antagonists for several hormone receptors, which are only a subset of all hormones. Many chemicals can act as EDCs in receptor-independent way, and there are an unknown number of chemicals for which the possibility that they are EDCs has not been evaluated yet [28]. Therefore, the whole picture of EDCs is likely to be much larger and much more complicated than what we currently imagine. Consequently, net effects of real world mixtures with a multitude of dissimilar modes of action in humans may be close to unpredictable [29,30].

2.2. Issue 2. Low reliability of exposure assessment

Direct measurement of chemicals or their metabolites in biospecimens is considered to be the most accurate method to assess exposure. However, many EDCs, especially EDCs with short half-lives and ubiquitous exposure sources, have innate limitations, which may make reliable exposure assessment impossible.

The intraclass correlation coefficient (ICC) is commonly used to describe the agreement of repeated measures over time within a subject. Nonpersistent chemicals with low ICCs demonstrate huge variability in repeated measures. For example, although BPA is the most well-known EDC, BPA has a particularly low ICC [31]. Even 24-hour urine samples did not estimate the usual exposure levels because of large day to day variability [32]. As a solution, multiple measurements or use of pooled samples are recommended [33], but the pooling of 35 repeated samples was estimated to be needed to reduce the attenuation bias of BPA to 10%

[34]. Other nonpersistent chemicals such as phthalate or polyaromatic hydrocarbons also demonstrate a low reproducibility, even though their ICCs are higher than those of BPA [35,36].

Despite this obvious impediment, human studies of chemicals with short half-lives have been increasing, and the low reliability of exposure biomarkers is dealt with simply as one study limitation. However, researchers need to ask themselves if human studies in which the exposure variable has intraindividual variation larger than interindividual variation can be excused by adding the statement of study limitation.

2.3. Issue 3. Nonmonotonic dose-response relationship and nonexistence of an unexposed group

Biological responses to EDCs do not necessarily increase with increasing dose of EDCs but often tend to plateau or even decrease, leading to nonmonotonic dose responses (NMDRs) [37]. NMDRs of EDCs can arise from numerous mechanisms such as opposing effects induced by multiple receptors differing by their affinity, receptor desensitization, negative feedback with increasing dose, or dose-dependent metabolism modulation [38]. The overcompensation of various adaptive responses through cellular stresses is also a mechanism leading to NMDRs [39,40].

Although evidence to date indicates NMDRs are often found in in vitro and mechanistic data, findings from epidemiological studies also support the occurrence of NMDRs within the environmental exposure range. For efficiency in the discussion, we propose several dose-response models (Fig. 1). Traditionally, potential risks at low doses are assumed to take one of two types of linear dose-response relationships: 1) linear threshold model (Fig. 1A) and 2) linear no threshold model (Fig. 1B). The linear no threshold model is standard for carcinogens, whereas the linear threshold model is standard for noncarcinogenic chemicals.

However, many environmental chemicals, both carcinogens and noncarcinogens, do not follow linearity within the low-dose range, but rather follow biphasic models more closely. In human studies, supralinear dose-response relationships have been suggested (Fig. 1C) [41]. Examples with rather consistent evidence are lead and IQ deficits [42,43], fine airborne particles and cardiovascular disease mortality [44], benzene and leukemia [45], and asbestos and mesothelioma [46,47]. Persistent organic pollutants (POPs) have recently emerged as a new risk factor for many types of obesity-related metabolic dysfunction, such as type 2 diabetes or dyslipidemia [48]. The associations between POPs and these diseases are also closer to supralinearity (Fig. 1C) in elders [49], but even the inverted U-shaped association (Fig. 1D) was observed in young people [50,51].

The bottom line of biphasic models is that the risk has a steep slope within the relatively low-dose range, slows down, flattens, or decreases as dose increases. This led to speculation that a greater biologic effect occurs at lower concentrations. This kind of nonlinearity observed in human studies has long been criticized as resulting from confounding, bias, or statistical artifact, rather than reflecting true biological mechanisms [52,53]. However, recent

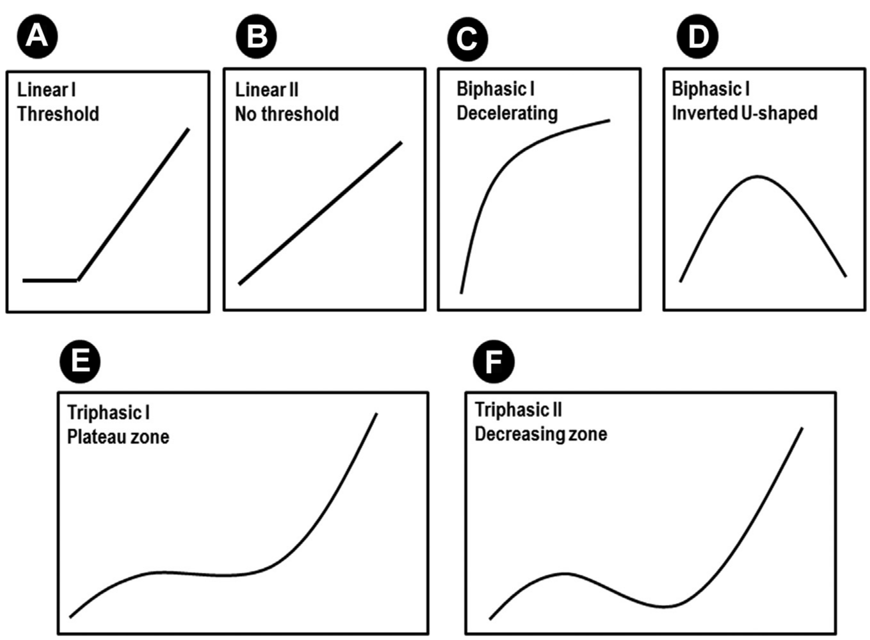

Fig. 1. Models of dose-response relationships. Linear models with/without threshold (A, B) are used in traditional risk assessment. However, biphasic with decelerating zone or decreasing zone (C, D) are possible within the environmental exposure range of chemicals. When a high-dose toxicity range is included, the whole dose-response curve may be closer to triphasic with plateau zone or decreasing zone (E, F). Within the low-dose range, interpretation of findings observed in human studies should always consider the possibility of chemical mixtures even though only one chemical is measured.

research about EDCs or hormesis suggests that the nonlinearity is biologically plausible.

Nevertheless, the whole range of the dose-response curve cannot be only biphasic because it is certain that extremely high-dose chemicals are harmful. We suggest that the whole range of the dose-response curve would be closer to triphasic (Fig. 1E and F). The extension of a decelerating biphasic curve to high-dose toxicity levels is shown in Fig. 1E (triphasic with a plateau zone), whereas the extension of the inverted U-shaped biphasic curve to high-dose toxicity levels is shown in Fig. 1F with a decreasing zone.

Notably, it is not easy to identify NMDRs in human studies, in particular when an unexposed group does not exist and the exposure range of the specific population under study is limited. As presented in Fig. 2, a variety of results, including positive, null, and inverse associations, are possible. Because of the omnipresence of EDCs, individuals without exposure do not exist for many EDCs. The most consistent positive association can be observed only among populations with a very low-dose range. These assertions are opposite to conventional wisdom in which any toxicity of chemicals can be best evaluated among populations with high-dose exposure.

2.4. Issue 4. Complicated interactions with known risk factors for chronic diseases

Many health behaviors are directly or indirectly related to the exposure to EDCs, metabolism and elimination of EDCs, or biological effects of EDCs at the cellular level. Therefore, when EDCs are investigated in human studies as possible risk factors, the adjustment for health behaviors as confounders may not be the right solution; health behaviors may be surrogate markers of the exposure to certain EDCs, epidemiologic mediators, or effect modifiers. In this article, we will focus on diet and obesity.

2.4.1. Diet

Diet is related to EDCs in multiple ways. First, food and food containers are the main exposure source of EDCs. For example, fatty animal food is one of the main exposure sources of lipophilic EDCs [54]. Chemicals like BPA or phthalate leach from food and beverage containers [55]. Pesticide residues in food and beverages also enter the human body. Furthermore, adverse effects of some EDCs tend to be observed among experimental animals consuming a high fat diet, but not a usual fat diet, suggesting biological interactions [56–58].

Second, many plant foods contain hormonally active substances. For example, isoflavones such as genistein possess powerful estrogenic activity in screening assays [59]. However, puzzling findings are that these natural EDCs can antagonize biological effects of man-made weak EDCs. For example, animal experimental studies demonstrated that genistein can antagonize effects of in utero exposure of BPA or phthalate exposure [60,61], even though the estrogenic potency of genistein is much higher than that of man-made xenoestrogens [62]. Often, bioassay such as the measurement of total xenoestrogen bioactivity is suggested as a possible approach for EDC mixtures

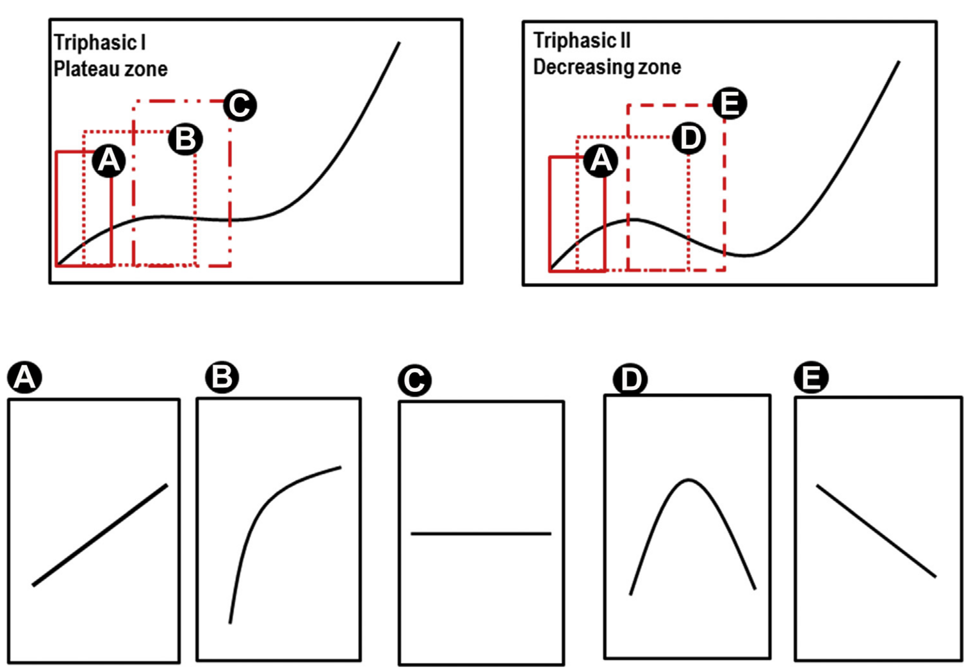

Fig. 2. Diverse results from epidemiological studies of endocrine-disrupting chemicals (EDCs). Depending on the range of exposure under triphasic dose-response relationships with plateau zone or decreasing zone, positive (A), supralinear (B), null (C), inverted U-shaped (D), or inverse associations (E) are possible. The most consistent positive association (A) can be observed when studies are performed within a population with low-dose range of chemicals.

[63]. However, antagonistic actions of phytoestrogens lead us to question the value of such a bioassay in human studies.

Third, many natural products demonstrate biological interactions with hormones or can be involved in metabolism of EDCs. For example, many components in foods such as flavonoids, stilbenes, carotenoids, and indoles bind the aryl hydrocarbon receptor [64] and they can show anti-estrogenic activity through aryl hydrocarbon receptor-estrogen receptor cross talk [65]. Nutrition can modulate the toxicity of EDCs [66]. Also, the various dietary fibers have the ability to absorb lipophilic EDCs such as POPs in the bile and increase their excretion in feces [67].

2.4.2. *Obesity*

At present, a popular research topic is whether exposure to EDCs during development can induce obesity [68]. However, the relation between EDCs and obesity is much more complicated than just obesity-inducing effects of EDCs because contemporary adipose tissue is an important internal exposure source of many EDCs, especially lipophilic ones [69]. The most well-known EDC class that is accumulated in adipose tissue is POPs [70]. Less lipophilic chemicals with brief half-lives, such as polyaromatic hydrocarbons, BPA, synthetic musk compounds, triclosan, and nonylphenol, are also detected in human adipose tissue [71,72]. In fact, the largest exposure source of EDC mixtures may be inside us, not outside us, because contemporary human adipose tissue contains the most complex EDC mixtures.

These chemicals in adipose tissue continuously release from adipose tissue through lipolysis. In particular, obesity with uncontrolled lipolysis and insulin resistance increases the chronic release of chemicals to circulation and can increase the chance that these chemicals reach critical organs [69]. Paradoxically, weight loss also leads to their release into the circulation, whereas weight gain can sequester them into adipose tissue [69]. The increased levels of POPs after weight loss are proportional to the amount of weight loss [73]. Thus, the toxicokinetics and toxicodynamics of POPs relate directly to the dynamics of adiposity.

On the contrary, adipose tissue can be seen as a protective organ against possible harms of POPs to other critical organs. For example, when two persons are exposed to the same amount of environmental POPs, the amount of environmental chemicals that reach critical organs will be less in obese than in thinner persons because the obese person has greater capability to sequester chemicals in adipose tissue. An animal experimental study clearly demonstrated the buffering effect of adipose tissue; the amount of adipose tissue plays a role as a modifier of the tissue distribution of lipophilic chemicals such as POPs [74].

All these complicated relationships between EDCs and obesity raise the question of how obesity should be treated in the epidemiology of EDCs. At present, obesity is considered to be an important risk factor for many chronic diseases. Although it is common in epidemiologic analysis to treat third variables as confounders, mediators, or effect modifiers, yet none of these approaches are a perfect fit in the relation between EDCs and obesity. In fact, most studies of obesity to date have considered the presence of EDCs in adipose tissue and the interconnected relationships between these two factors.

3. Can exposome be a solution?

Despite the many methodological issues in human studies of EDCs, there is an optimistic prospect that innovative breakthroughs in biomedical research encompassing the omics fields, advances in mobile sensing, and statistical analyses of big data can provide tools, which can help assess the role of EDCs in the risk of human disease [75,76]. This concept has been called the "exposome" in which the target is to gather information about the totality of exposures across the life span [77,78]. However, despite this fascinating concept, the exposome is never free of the most fundamental methodological limitations previously discussed.

Among various omics, only genomics are stable; all other omics are dynamic. Namely, epigenomics, transcriptomics, proteomics, and metabolomics are continuously affected by ever-changing external and internal environments [79]. In addition, although many sophisticated statistical methods have been suggested for analyzing huge data sets derived from the exposome [80,81], it is perhaps ironic that they presume unrealistic simple conditions, such as linearity.

4. Possible research topics

Human studies of environmental chemicals with long half-lives continue to be worthwhile. The example is POPs. Even though human studies of POPs also suffer from some inconsistencies, they are at least more consistent than those of common EDCs with short half-lives [13,82]. In fact, human studies of POPs, especially chlorinated POPs, are less affected by methodological issues because of their long half-lives, low-dose exposure range, and the presence of a reference group that is close to unexposed. Furthermore, there is another important aspect of POPs in human studies; serum concentrations of several POPs play a role as surrogate markers of lipophilic chemical mixtures, including both those that are measured and those that are unmeasured.

Furthermore, the dynamics of POPs during weight loss can provide a unique opportunity to study harmful effects of POPs. The release of POPs from adipose tissue to circulation during weight loss was related to harmful effects on resting metabolic rate, sleeping metabolic rate, thyroid hormone levels, and the oxidative potential of skeletal muscle [83−85]. Even though the net improvement of cardiometabolic risk factors is well known among weight losers with metabolic diseases [86], a tradeoff effect between benefits of decreased fat mass and harms because of POPs

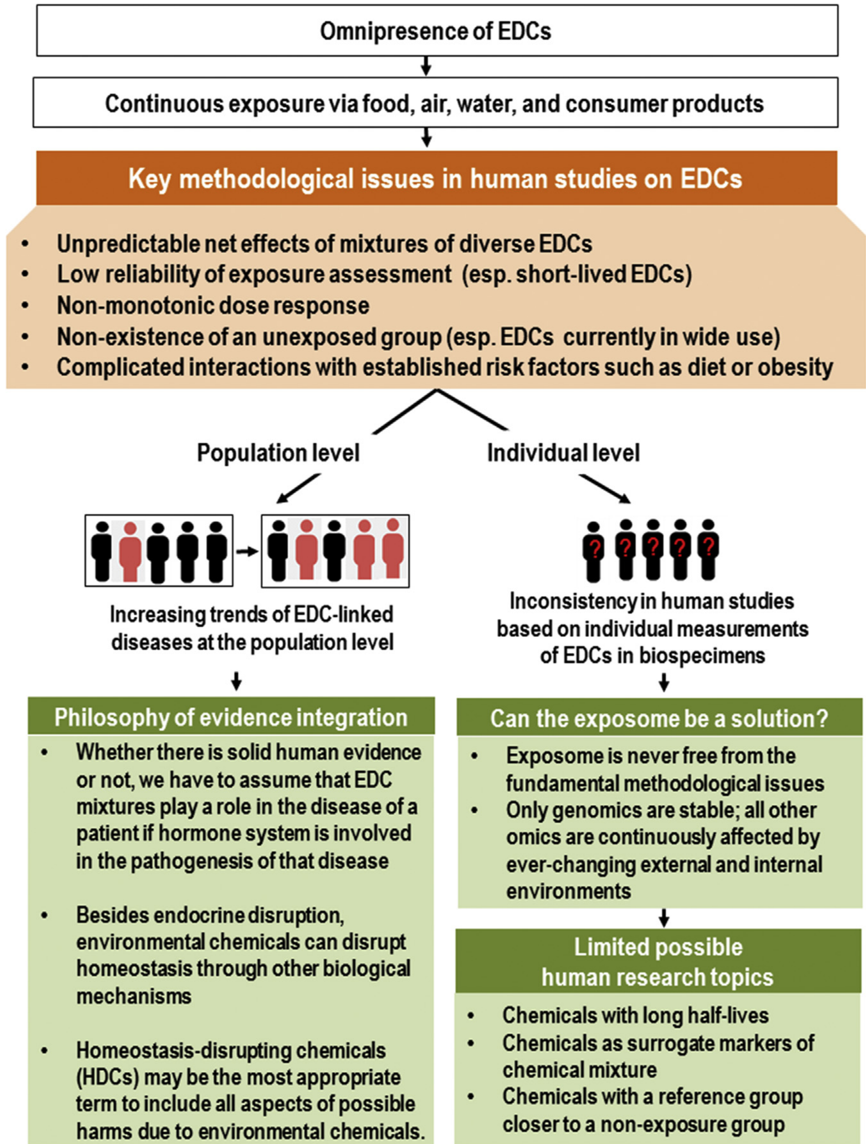

Fig. 3. Issues in human studies of endocrine-disrupting chemicals (EDCs). Despite the increasing trend of EDC-linked diseases, it is difficult to find consistent human evidence about the harms of EDCs, especially concerning the short-lived EDCs currently in wide use because of critical methodological issues. In the case of human studies, possible research areas are limited, and the philosophy of evidence integration should be used to judge harms of EDCs in humans.

released from adipose tissue to circulation needs to be carefully evaluated.

POPs are well-known EDCs [87,88]. However, it would not be appropriate to attribute human findings about POPs to their traditional hormone-disturbing properties because POPs are a mixture of diverse EDCs. Again, the net effect of EDC mixtures that can act as agonists or antagonists to multiple hormones that cross talk cannot be reliably predicted, despite the reliable exposure assessment of POPs. Other mechanisms, such as mitochondrial dysfunction, have been suggested as possible mechanisms linking POPs to health outcomes [48,89].

Besides endocrine disruption, environmental chemicals can disrupt homeostasis through other biological mechanisms. Therefore, focusing on only hormone-disturbing properties of chemicals may itself be short sighted. Recently, new terms such as metabolism-disrupting chemicals (MDCs) [90] or mitochondrial function–disrupting chemicals (MtDCs) [91] have been coined. A single chemical can be correctly called EDC, MDC, or MtDC depending on context. However, we suggest homeostasis-disrupting chemicals (HDCs) to include all aspects of possible harms because of environmental chemicals. EDCs, MDCs, or MtDCs would then be regarded as subsets of HDCs.

5. Conclusion

The whole concept of this article is portrayed in Fig. 3. The most persuasive evidence about harms of EDCs in humans may be an increasing trend of EDC-linked diseases at the population level. However, traditional epidemiological studies based on individual measurements of EDCs, especially many short-lived EDCs currently in wide use, may fail to provide consistent results for the methodological reasons discussed aforementioned. As these limitations are inherent, they may not be overcome by innovative technology even in the future.

Therefore, it should not be inferred from inconsistency in findings among traditional epidemiological studies that the chemicals studied have been proven not to be harmful in humans. Regarding EDCs, the philosophy of evidence integration should be used [2]. When high-quality studies demonstrate that a chemical interferes with hormone action in vivo and in vitro at concentrations relevant to humans, and these hormone systems are essential in physiological functions of certain organs, it is reasonable to infer that chemicals that affect these hormone systems will produce adverse effects in humans. Epidemiologists who want to investigate the role of EDCs and other chemicals in human health need to clearly recognize the methodological issues related to EDCs. Finally, it is important to note that, even if an adverse effect of EDCs cannot be definitely proven in human studies, there may be no health-related risk to restricting these chemicals.

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