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Endocrine disruptors

Endocrine disruptors: Revisiting concepts and dogma in toxicology

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ABSTRACT

During the last decades, a large number of observations have shown that some exogenous substances could interfere with hormone levels or hormone action and could induce toxic effects. This has led to the identification of endocrine disruptors more than 25 years ago as a new class of toxic agents (Zoeller et al., 2014). Those widely used agents correspond to a variety of chemical classes, are not identified by their chemical structure or by a specific type of usage, but rather by their mechanisms of action; this is not unprecedented in toxicology since genotoxicants have also been identified by their mechanism of action, i.e. their ability to alter DNA structure and function.

During the last decades, a large number of observations have shown that some exogenous substances could interfere with hormone levels or hormone action and could induce toxic effects. This has led to the identification of endocrine disruptors more than 25 years ago as a new class of toxic agents [1]. Those widely used agents correspond to a variety of chemical classes, are not identified by their chemical structure or by a specific type of usage, but rather by their mechanisms of action; this is not unprecedented in toxicology since genotoxicants have also been identified by their mechanism of action, *i.e.* their ability to alter DNA structure and function.

It is not overstated to claim that the discovery of EDCs has opened a new era in the field of toxicology. Studies on EDCs have questioned some of the dogma of traditional toxicology and have changed our ways of viewing toxic actions. In this short report, we will show how those chemicals have triggered both conceptual and practical changes in our approach to toxicity.

1. Back to physiology

One of the most striking consequences of EDC work is that toxicology and physiology have been brought together again. For those interested in the history of toxicology, it is interesting to recall that the initial development of this science in the 19th century has been carried out by physiologists and medical scientists such as Claude Bernard and François Magendie [2]. This was indeed critical to understand the systemic effects of certain toxicants such as curare. However, other disciplines have considerably influenced toxicological studies. Indeed, toxicology has been intimately associated with analytical sciences, which allowed basic and regulatory scientists to detect and quantify toxicants and to answer critical questions such as the type of exposure to chemicals and its level. Later, toxicologists started using chemical, molecular and cellular concepts and tools, and were able to develop mechanistic approaches. To a certain extent,

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this has shifted the focus away from physiological approaches. However, with the advent of EDCs, toxicologists started to address such questions as the consequences of subtle changes in sex hormone levels during the menstrual cycle or the effect of exposure to contaminant during the critical phases of organ development. Clearly, a more integrated approach was required and therefore, toxicologists had to go back to their physiology textbooks. This is not all. In fact, disruption of the endocrine system is only one aspect of what exogenous substances can do to alter a physiological system. There are clearly substances that interfere with the nervous system and not necessarily by disrupting hormone action. Other substances could change developmental programming through mechanisms that are not necessarily related to endocrine action. Those substances share with EDCs a number of properties and consequently we tend to call them EDCs too, although this is not strictly correct. What we are actually talking about are Physiological Homeostasis Disrupting Compounds (PHDCs). Now, the "EDC" brand name is well established and it is probably too late to change, but we have to acknowledge that we deal with EDCs and EDC-like compounds. This has regulatory implications since the EU definition is strictly related to endocrine disrupting compounds and would not cover compounds that may interfere with the nervous system or the immune system.

2. The dose

The discovery of the EDCs has added another chapter to the "low dose" issue in toxicology. This is not a new issue. The 1950s and the 1960s have witnessed some strong controversies between different groups of toxicologists. Some claimed that for most compounds there was no safe dose, even at low concentrations, while other claimed that, below a certain threshold that should be determined, most compounds were safe. The latter view prevailed but with the important exception of genotoxicant carcinogens for which it was considered that even very low doses could lead to irreversible effects (*i.e.* mutagenesis and long-term effects). This Yalta-like conclusion was the dawn of regulatory toxicology and of regulatory reference values below which compounds were considered essentially harmless. It should be noted that scientific foundations for the calculation of those reference values are at best controversial. EDCs have brought the dose issue to light again. First, it was observed that low doses, *i.e.* doses similar to a usual environmental contamination, can have significant effects in some experimental models, notably during developmental windows of vulnerability [3]. This means that some reference values that are determined based on regulatory and often non-comprehensive tests may not be protective enough. One has to keep in mind that most toxic effects related to EDCs have not been discovered through traditional regulatory tests, but rather by academic scientists exploring new mechanisms of toxicity. The second important point is that, in some cases, the dose response curve describing one toxic effect of a chemical as a function of its dose may not be monotonous. Intuitively, most of us would think that a toxic effect should increase with the dose. In reality, there are cases where effects are more potent at lower doses than at higher ones. This has been discussed at length in several conferences and papers [3,4]. The mechanisms are diverse and could be related to multiple mechanisms of action triggered at different doses or to the intrinsic properties of the endocrine system. Recently, Anses and other EU agencies have critically analyzed the literature for dose effects and concluded that, while some of the claims for non-monotonous dose response curves are overstated, there are indeed a few cases where dose response curves could confidently be considered as non-monotonous in humans [5]. One important consequence of non-monotonous curves is that regulatory tests should now encompass a much larger dose range than previously in order not to miss a specific low dose effect and that identification of reference doses may become even more difficult than in the past [6]. A reevaluation of the regulatory approaches to reference value determination appears to be required.

3. Time

One of the most challenging tasks in toxicology is to understand the mechanisms of long-term effects leading to chronic diseases and to find the right models to study them. Long-term means years, decades and possibly generations! With the exception of mutagens, long-term effects were traditionally thought to be related to continuous exposure as in the case of air pollution and smoking. Toxicity related to long-term continuous exposure has some paradoxical features. Indeed, in many cases such a long-term toxicity is unexpectedly related to the adaptive metabolic pathways that are triggered by exposure to chemicals; those pathways, by allowing the elimination of chemicals are protective in the short term, but they also entail the transient production of very reactive intermediate compounds that may lead to toxicity in the long run [7]. What this is telling us is that the same pathway could be adaptive or toxic depending on the time scale that is considered. Long-term effects could also be due to the internal persistence of chemicals as in the case of Persistent Organic Pollutants (POPs) which are poorly metabolized and eliminated and which are stored in adipose tissue; the latter, in turn, becomes an internal source of continuous exposure [8]. Again, this tissue has a paradoxical effect toward POP handling; by storing those pollutants, the adipose tissue protects other sensitive organs such as the brain or gonads, but in the long run, it does constitute an internal source of chronic exposure.

With EDCs, a third mechanism was unraveled. Indeed, both experimental and epidemiological studies indicated that exposure to several EDCs at specific developmental stages was associated with an increase in the risk of disease later in life [9]. In that case, exposure can be either continuous or limited in time, but the targeted organism is in a state of high vulnerability. It is thought that vulnerability is due to the remodeling of tissues and organs during development and to limited defense mechanisms. The most likely mechanism is through the alteration of epigenetic marks that are somatically heritable and that therefore may persist for a long time [10]. Such alterations

Table 1
What EDC studies have brought to concepts and properties in toxicology.

Concepts and features	Traditional toxicology	EDC inspired toxicology	Implications
Dose-response	Monotonous with or without threshold Tests at relatively high doses	Monotonous or non-monotonous Low-dose effect	Identify mechanisms of non-monotonous effects Test a large range of doses
Long-term effects	Continuous exposure Persistence	Continuous exposure Persistence Programming: delay between exposure and effects	Challenge to test programming Focus on epigenetics
Windows of developmental vulnerability	Not distinguished from continuous exposure	Highly relevant and mechanistically distinct from continuous exposure	Should be tested at least for confirmation Epigenetic marks may constitute exposure and effect biomarkers
Mixture effects	Ignored	Highly relevant particularly for similar modes of action Other interactions should be explored	Should be tested at least to confirm dose addition when similar MoA
Interaction with other stressors	Ignored	Relevant in particular when the hypothalamo pituitary axis is targeted	Studies should take into consideration combined stressor effects (exposome)

could lead to subtle changes in organ physiology that may increase the risk of disease development later in life. As an illustration, altered methylation of adipose tissue genes that are targets of the metabolically critical receptor PPARγ by certain EDCs are thought to increase the risk of adiposity in mice [11]. We still need more data linking EDC-triggered epigenetic regulation to health effects. We also need to develop the concept further and to weigh the evidence supporting the similarity between epigenetic toxicity and genotoxicity. Indeed, both can account for long-term effects, although epigenetic marks are reversible in the long run, which is not the case of mutations. Regulatory implications are important in that epigenetic toxicity may fall in the "no-threshold" toxic effect category.

4. Vulnerability of the target

Whereas traditional toxicology has primarily focused on the substance ("the dose makes the poison"), it has now become clear that the state of the targeted organism is also critical. Indeed, under certain conditions, organisms can be more vulnerable to the toxicity of chemicals [12]. This could be due, for example, to the genetic background of the contaminated individual, which has led to the development of gene–environment interactions in population studies. Another source of vulnerability is the health and social status, for example if the individual suffers from diabetes, kidney disease, or low socio-economic status. But, as stated earlier, most of the recent advances have been made on the developmental stage, since certain periods of development display a higher likelihood of toxicity in case of exposure to a chemical. Clearly, the extensive epigenetic remodeling and stem cell differentiation during development is a likely cause of vulnerability particularly when toxicity is related to a modification of programming [12].

5. The mixture effect

Interest in mixture effects precedes the discovery of endocrine disruptors. It is in fact a traditional issue in pharmacology and toxicology and is particularly relevant when the identification of toxicants is related to the mode of action. For example, several dioxins, furans and PCBs share a similar mechanism of action, i.e. the activation of the ArylHydrocarbon Receptor, albeit with different potencies [13]. In addition, those compounds, which are highly lipophilic, tend to accumulate together in fat, so that co-contamination is very frequent. The toxic equivalency concept was developed to account for such combined exposures and the dose addition method was developed accordingly. Because EDCs are also identified by their mechanism of action, it was also relevant to classify them accordingly. Studies have focused on those that display a similar mode of action, for example anti-androgenic effects or xeno-estrogenic effects and, in that case, the current evidence suggests that dose addition should be used to account for mixture effects [14]. The study of the combination of EDCs with different mode of action is less advanced; authors have found different outcomes such additive, synergistic or antagonistic effects. This is a major challenge for the near future with implications in both basic and regulatory sciences.

EDCs have constituted a unique stimulus pushing toxicological concepts forward (Table 1). Together with the exposome concept [15], the endocrine disruption concept has led to a more systemic approach to toxicity, combining environmental sources of exposure, health effects and an integration of different stressors (chemical physical, biological, psychological, social) during the lifetime. There are implications both in basic sciences, since we are witnessing increasing links between toxicology, physiology, developmental biology and epigenetics as well as in regulatory sciences, which are considerable. The latter will probably change the landscape of regulation in the EU and worldwide. This is indeed a wonderful example of the challenges and opportunities of translating science into policy.

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