

## FORUM

## Principles of Pharmacology and Toxicology Also Govern Effects of Chemicals on the Endocrine System

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### ABSTRACT

The present debate on chemicals with Hormonal activity, often termed 'endocrine disruptors', is highly controversial and includes challenges of the present paradigms used in toxicology and in hazard identification and risk characterization. In our opinion, chemicals with hormonal activity can be subjected to the well-evaluated health risk characterization approach used for many years including adverse outcome pathways. Many of the points arguing for a specific approach for risk characterization of chemicals with hormonal activity are based on highly speculative conclusions. These conclusions are not well supported when evaluating the available information.

**Key words:** endocrine disruptors; endocrine toxicology; risk assessment; regulatory/policy; risk assessment.

The potential impact of environmental chemicals on the endocrine system in humans is an area of intensive and controversial debate. In this context, scientists from the area of endocrinology have claimed that approaches to hazard assessment widely accepted in toxicology are 'outdated' and have questioned the procedures applied in health risk assessment by regulatory agencies worldwide (Myers *et al.*, 2009; Vandenberg

*et al.*, 2009, 2010, 2012). This debate has resulted in proposed regulations in Europe based on the 'precautionary principle' and in discussions on classification of chemicals with hormonal activity as 'endocrine disruptors'. As pointed out in the 2002 WHO/IPCS report and by the Scientific Committees of the European Commission (CSTEE, SCHER) 'endocrine disruption' is not a toxicological endpoint *per se*, but one of the many modes-of-action

which may result in adverse effects. Accordingly, modes-of-action should not be used as a basis for classification and labeling of chemicals, and the special classification of a chemical as 'endocrine disruptor' is inconsistent with the established and proven procedures of chemical classification and labeling, and thus should be avoided.

In a recent editorial (Dietrich *et al.*, 2013) published in several toxicology-oriented journals and in an open letter (Open-Letter, 2013) to the then Chief Scientific advisor of the EU commission, Prof. Anne Glover, many colleagues have already expressed their concerns regarding the proposed inappropriate regulation. The letter was signed by numerous researchers and teachers in pharmacology and toxicology. Many of them also have been active in advisory groups charged with risk assessment and/or participated in expert teams for the topic of 'endocrine disruption' at the level of the European Union or the OECD. In response to this editorial and the open letter (Dietrich *et al.*, 2013; Open-Letter, 2013), several articles and press releases were published in favor of hazard-based regulation and classification of 'endocrine disruptors' (Garwood, 2014; Gore, 2013; Grandjean and Ozonoff, 2013; Horel and Bienkowski, 2013). Many of these articles contained foremost irrelevant *ad personam* attacks and included only a limited scientific discussion of the issues that were raised.

Aspects of approaches to health risk assessment for 'endocrine disruptors' (Borgert *et al.*, 2013; Dekant and Colnot, 2013; Dietrich, 2010; EU-SCCS, 2014; Greim, 2005; Hengstler, 2014; Open-Letter, 2013; Testai *et al.*, 2013) have been published. Since the debate remains controversial and apparently in deadlock, we wish to expand the discussion of the approaches to health risk assessment for 'endocrine disruptors' into the toxicology community. A new article titled 'A path forward in the debate over health impacts of endocrine disrupting chemicals' (Zoeller *et al.*, 2014) proposes a 'way forward' in the discussion regarding potential health impacts of 'endocrine disruptors'. The proposal by Zoeller *et al.* (2014) to engage in a rational debate based on principles of science is highly appreciated since a pathway forward is needed.

However, the Zoeller *et al.*'s proposal to delineate a scientifically sound way forward is again unnecessarily flawed by an imbalanced presentation of available information and some misinterpretation of statements. For example, while Zoeller *et al.* (2014) mention that the report published by WHO/UNEP (WHO/UNEP, 2012) was criticized, they neither mention who criticized this report nor the reasons for the critique. The critique (Lamb *et al.*, 2014) was developed by a group of experts including some of the main authors of the first WHO/UNEP (WHO/UNEP, 2002) report. Their main concerns regarding the WHO/UNEP (2012) report were:

- The 2012 WHO/UNEP report does not follow the weight-of-evidence approach recommended by the 2002 WHO/UNEP report and presents data and controversial topics (i.e. low dose effects, nonmonotonic dose response) in an unbalanced way.
- In the 2012 report, 'endocrine disruption' is often postulated to occur based on an exposure assessment or a potential mechanism for 'suspect' chemicals despite a lack of support for a causal relationship as requested by the definition of 'endocrine disruption'.
- In the 2012 report, a causative role of 'endocrine disruption' is often inferred by combinations of a series of unrelated facts, which collectively do not demonstrate causation.
- In the 2012 report, 'endocrine disruption' is implicated as the basis for trends in disease incidence or prevalence without adequately considering other potential risk factors.
- Basic principles of dose and potency are often ignored.

Unfortunately, Zoeller *et al.* (2014) again do not provide a balanced approach of available data in their proposal. Scientists in the field of toxicology, pharmacology, and risk assessment are striving to provide for a weighing of appropriate evidence to allow a realistic evaluation of health risks for humans (EFSA, 2013; EU-SCCS, 2014). As emphasized earlier (Dietrich *et al.*, 2013) and corroborated later (Lehman-McKeeman and Kaminski, 2013), scientists owe it to their scientific integrity to provide the best evaluation of data possible. Therefore, the 2002 WHO/UNEP report demanded that a review of all data on endocrine disruption is to be appropriately performed according to the well-established principles of data evaluation. This was not adequately performed in the WHO/UNEP report of 2012 and is also missing in the Zoeller *et al.*'s (2014) article.

Regarding the views expressed by Zoeller *et al.*, we would like to comment on aspects that remain controversial:

- As in previous papers, Zoeller *et al.* present the endocrine system as a unique biological system, which should require special considerations in hazard identification and risk characterization that are not relevant when assessing other somatic targets for toxicity. However, basic research in pharmacology and toxicology on endocrine active compounds as well as decades of clinical experience—e.g. the use of contraceptives and hormones in the treatment in osteoporosis—show that this is not the case. The extensive database on the outcome of prenatal diethylstilbestrol (DES) exposure (reviewed by Golden *et al.*, 1998; Hoover *et al.*, 2011) show a high degree of concordance between effects observed in humans and in rodent models. Moreover, animal studies with perinatal exposure to DES are consistent with dose-related effects for adverse outcomes (Dietrich, 2010). This is also documented for other potent drugs, such as tamoxifen and antiandrogens (Iguchi *et al.*, 1986; Imperato-McGinley *et al.*, 1992; Newbold *et al.*, 1997).
- Interference of a chemical with hormone-mediated pathways is one of many possible modes of action resulting in adverse (or therapeutic) effects after chemical exposures. The many other modes-of-action elucidated by mechanistic toxicology also include potential windows of susceptibility, potentially sensitive subgroups, complex mechanisms, and often many mechanistic steps with limited understanding (Klaassen, 2013). Therefore, well-planned and conducted research toward a better understanding of all modes of actions responsible for toxicities of chemicals is needed. Only on such a basis, can we integrate the presently available modes of action into reasonable risk assessment approaches to support causality (Carmichael *et al.*, 2011; Dekant and Colnot, 2013; Testai *et al.*, 2013).
- Many of the known modes of action in toxicology include receptor-mediated mechanisms, e.g. chlorinated dioxins, many chemicals acting as enzyme inducers, and many chemicals interfering with neurotransmission. In addition, modern toxicology has developed largely from studies of drug safety and safety assessment of medicines remains one of the major fields of toxicology. Numerous drugs act through cellular receptors. Thus, there is abundant information on the principles of receptor interactions, which is often ignored in the dispute on 'endocrine disruptors'. Indeed, relevant and longstanding state-of-the-art experience is available in receptor-mediated effects in toxicology and pharmacology.
- The limitations of epidemiological data in health risk characterization regarding environmental exposures are the low sensitivity of epidemiology, issues with study design, exposure assessment, multiple endpoints of potential relevance, and the presence of confounders (Mirmira and Evans-Molina, 2014).

Yet, careful follow-up of the DES-exposed cohorts was able to provide cumulative risk estimates for several adverse outcomes which had been documented in females and males (Hoover *et al.*, 2011; Palmer *et al.*, 2009). However, DES is a highly potent estrogen and was applied in high doses during pregnancy (Dietrich, 2010). In contrast, doses of chemicals with potential hormonal activity received from the environment are very low and most of these compounds have a very low potency (EFSA, 2015; Safe, 1995,2000).

- We agree with the conclusion regarding the WHO/IPCS definition for adversity as useful basis for further discussions, but also support the WHO/IPCS definition for an 'endocrine disruptor' as does EFSA and SCCS (EFSA, 2013; EU-SCCS, 2014). A logical consequence of using these definitions is that potency is expressed by a benchmark dose (or NOAEL) and is derived from the dose-incidence curve for adverse effects in intact animals from appropriate toxicity studies. This has been integrated in potency assessments for both synthetic and natural chemicals for decades. Apparently, there is agreement on the use of NOAELs or benchmark doses as indicators of potency in risk assessment, and, in fact, one of the authors of the Zoeller's article (2014) has used NOAELs and benchmark doses to assess relative potency in cumulative risk assessments (Kortenkamp and Faust, 2010).
- Using adverse effects in intact animals for hazard assessment will also permit to identify chemicals with hormonal activity where the parental compound does not show interactions with hormone receptors, but where metabolites, often formed by complex pathways, are hormonally active and may cause adverse effects (Reinen and Vermeulen, 2015; Reinen *et al.*, 2011; van Liempd *et al.*, 2006).
- Theoretical considerations and decades of experience with numerous chemicals from many structural classes with many different modes of action provide strong evidence of thresholds (Borgert *et al.*, 2013). The existence of thresholds is plausible since endogenous hormones are active in the presence of a  $10^5$  to  $10^9$  M excess of other endogenous chemical constituents. Many of these other endogenous chemicals have low affinities to hormone receptors or even have some marginal intrinsic activity. When the affinity of an endocrine active chemical toward a specific receptor and its internal concentration are orders of magnitude lower compared with physiological concentrations of respective hormones, they are very unlikely to cause adverse effects via this specific receptor.
- Regarding the mechanistic interpretation of Zoeller *et al.* (2014) for 'endocrine disruption', the authors focused on the potential influence of an 'endocrine disruptor' on hormone 'action' which they equate to 'hormone receptor activation'. This approach is inconsistent with the way toxicology and generally accepted risk characterization procedures describe toxicologically relevant processes in terms of an adverse outcome pathway (OECD, 2013). Zoeller *et al.* seem to imply that any direct or indirect interaction with hormone action should be considered as adverse. In reality, by applying an adverse outcome pathway approach, such interactions can only be regarded as possible molecular initiating events. Zoeller *et al.* disregard the further description of events leading or not leading to a possible adversity toward the functionality of the integrated systems.
- Guideline studies following OECD, US EPA, and European regulations, or performed following guidance by the International Committee on Harmonization for pharmaceuticals, are established tools for hazard identification. Studies following such guidelines determine a variety of endpoints related to adversity. A wealth of experience exists regarding the interpretation of

results from such studies and their integration into risk characterization. In addition, detailed reporting of raw data from such studies is available and well-evaluated and validated methods are used for all determinations. We are aware that the guideline studies have limitations and represent compromises. We agree that, from a scientific view, it may be desirable to expand such studies and additionally include a variety of molecular endpoints, which is already performed in some studies, such as the 'BPA-Clarity' project (Birnbaum *et al.*, 2012). However, it remains to be demonstrated whether these additional molecular endpoints are more sensitive than the adverse effects determined in guideline studies and more importantly, whether they can be extrapolated to humans.

- The point made by Zoeller *et al.* that nonguideline studies are not considered in risk assessments is not valid. The US EPAs IRIS assessments, REACH dossiers, EFSA evaluations, and most others risk assessments strive to use all available data including mechanistically oriented nonguideline studies. An assessment of reliability of studies, consistency of the database, and a weight-of-evidence approach in the evaluation of inconsistent databases (EFSA, 2015) is established in hazard and risk assessment worldwide and was specifically embraced by the WHO/UNEP report on 'endocrine disruption' in 2002.
- The comments about differences between adverse effects between synthetic and natural chemicals have no scientific basis. Toxicity is governed by chemical structure and the possibility of a chemical to interact with biological systems, but not its origin. When appropriately tested, toxicity profiles of hormonally active natural compounds are very similar to those of industrial chemicals with some hormonal activity (Belli *et al.*, 2010; Delclos *et al.*, 2009; Latendresse *et al.*, 2009; Tyl *et al.*, 2008a, b; Zhao *et al.*, 2013). Interestingly, many natural chemicals (e.g. isoflavones, steroids, zearalenones) are more potent than the typical industrial chemical with consumer exposures. Thus, just like for industrial chemicals, these compounds produced by nature also need to be subjected to the process of a scientifically based risk assessment.

In conclusion, appropriately designed and conducted toxicity studies must include determination of the potency by which a chemical induces adverse effects. This inherently includes potential adverse effects on the endocrine system, which could manifest as reproductive and developmental toxicities. Whenever such toxicities are identified based on well-developed and robust endpoints, this will result in the classification of the respective chemical as a 'Reproductive/Developmental Toxicant'.

We strongly support a rational debate based on factual data and established principles of science and specifically of pharmacology and toxicology. Such principles remain relevant to assess potential health impacts of chemical exposures, and only the application of such principles will permit a path forward in discussing the potential health impacts of 'endocrine disruptors'.

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