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Risk assessment of 'endocrine substances': Guidance on identifying endocrine disruptors

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HIGHLIGHTS

- A scientific approach to identifying endocrine disruptors of concern for human health.
- Shows examples of endocrine disrupting chemicals of different levels of concern.
- Explains the scientific evidence required to regard a chemical as an endocrine disruptor.

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ABSTRACT

The European regulation on plant protection products (1107/2009) and other related legislation only support the marketing and use of chemical products on the basis that they do not induce endocrine disruption in humans or wildlife species. This legislation would appear to make the assumption that endocrine active chemicals should be managed differently from other chemicals presumably due to an assumed lack of a threshold for adverse effects. In the absence of agreed scientific criteria and guidance on how to identify and evaluate endocrine activity and disruption within these pieces of legislation, a European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) task force was formed to provide scientific criteria that may be used within the context of these three legislative documents. The first ECETOC technical report and associated workshop, held in 2009, presented a science-based concept on how to identify endocrine activity and disrupting properties of chemicals for both human health and the environment. Specific scientific criteria for the determination of endocrine activity and disrupting properties that integrate information from both regulatory toxicity studies and mechanistic/screening studies were proposed. These criteria combined the nature of the adverse effects detected in studies which give concern for endocrine toxicity with an understanding of the mode of action of toxicity so that adverse effects can be explained scientifically. A key element in the data evaluation is the consideration of all available information in a weight-of-evidence approach.

Both sets of data (evidence of the adverse effect in apical studies and conclusive mode of action knowledge) are essential in order to correctly identify endocrine disruption according to accepted definitions. As the legislation seeks to regulate chemicals on a mode of action rather than the more traditional approach of adverse endpoints, then conclusive evidence of the mode of action of concern should be presented. From a human safety perspective and in the absence of any compelling data that endocrine active chemicals exert their adverse effects through anything other than a threshold mechanism there is no scientific justification for not using a margin of exposure approach to risk assessment in order to best protect human health.

Introduction

Recent European legislation (Plant Protection Products Regulation 1107 [EC, 2009]) has created a hazard based approval criterion that only supports the marketing and use of chemicals on the basis that they do not induce 'endocrine disruption' in humans or wildlife species. This legislation would appear to make the assumption that endocrine active chemicals should be managed differently from other chemicals presumably due to an assumed lack of a threshold for adverse effects. Moreover, the fundamental scientific criteria necessary to define endocrine disrupting properties, are not described in any of these legislative texts or accompanying guidance. Substances which interfere with the endocrine system are often referred to as 'endocrine disruptors'. While this may be

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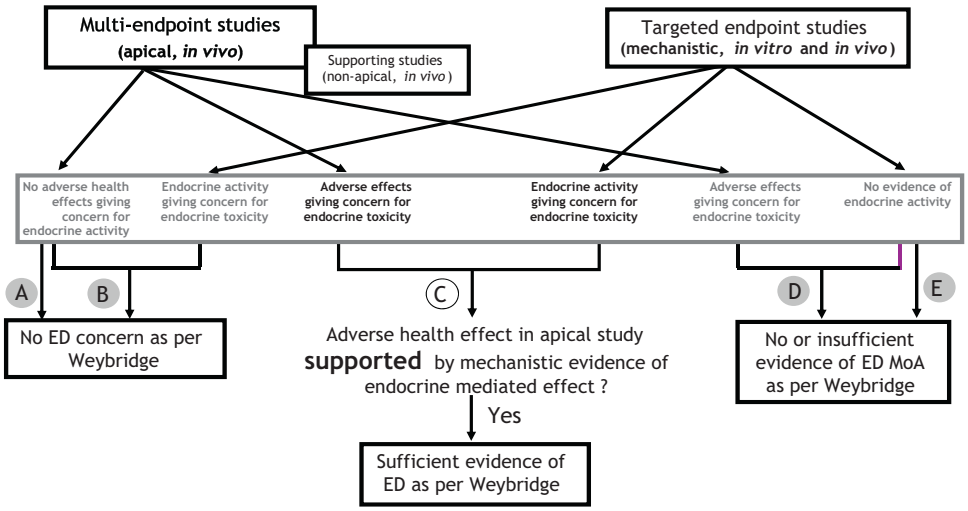

Fig. 1. Flow chart for effects in mammalian species.

considered an inappropriate terminology by some, it has become common practise and is therefore used occasionally in this paper.

The ECETOC approach

In response to these legislative developments and in the absence of scientific or any other criteria, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) formed a task force to develop a science-based proposal on how to identify and assess chemicals with endocrine disrupting properties (ECETOC, 2009a) and shared this approach at a tripartite (industry, regulatory authorities and academia) workshop (ECETOC, 2009b). The proposed guidance was refined and published (Bars et al., 2011a). The proposed scientific criteria integrated, in a weight of evidence approach, information from regulatory toxicity studies and mechanistic/screening studies. These criteria combined evidence for adverse effects detected in apical whole-organism studies with an understanding of the mode of action (MoA) underlying the adverse events. The first part of the guidance consisted of flow charts describing data combinations of evidence (or absence thereof) that would lead to the determination that a substance had endocrine disrupting properties (Bars et al., 2011b). In addition, since not all chemicals with endocrine disrupting properties are of equal hazard, an assessment of potency was also proposed as a second step to discriminate chemicals of high concern from those of lower concern (Bars et al., 2012).

The ECETOC approach is consistent with the approaches proposed by individual EU member states for determining endocrine disrupting properties (BfR, 2011). The current paper presents the original concepts of the ECETOC guidance, specifically the need to apply a rigorous weight of the evidence approach with supporting evidence based MoA knowledge.

The criteria proposed by the ECETOC task force (ECETOC, 2009a; Bars et al., 2011a) were based on two key elements that need to be fulfilled and they are consistent with the broadly accepted definitions for endocrine disrupting chemicals (*e.g.* Weybridge, 1996; EC, 1999; IPCS, 2002; Japanese Ministry of the Environment, 2005), namely that exogenous substances need to cause adverse effects in intact organisms and that the adverse effect is caused by an endocrine MoA. It is therefore clear that, for a chemical to fulfil the criteria and be identified as an endocrine disruptor, there should be evidence of adverse effects in the apical studies listed below and that there should be convincing evidence from MoA studies that the adverse effect is a consequence of a perturbation of normal endocrine control. An adverse effect in apical studies alone or evidence of endocrine perturbation in short term mechanistic studies but without evidence of adverse effects in standard bioassays is not sufficient to fulfil the criteria that identify a chemical as an 'endocrine disruptor'.

The current primary toxicology test methods for detecting endocrine toxicity in mammals are the standard regulatory OECD test guideline studies including the rodent two-generation reproduction study (TG 416), the extended one-generation reproductive toxicity study (TG 443), the rodent chronic toxicity and oncogenicity studies (TG 451, TG 452, TG 453), and the recently enhanced 28-day toxicity study (TG 407). Evidence for the MoA is best provided by (but not limited to) the recently validated *in vitro* and *in vivo* screening studies included in the US EPA Tier 1 endocrine test battery or levels 2 to 4 of the OECD conceptual framework for the testing and assessment of endocrine disrupting chemicals.

The original ECETOC guidance considered five scenarios to guide the evaluation of available mammalian data to determine whether a substance has endocrine properties (Bars et al., 2011a; Fig. 1). Only one scenario (Scenario C) describes the data combination that would result in the conclusion that there is sufficient evidence of endocrine disruption. This data combination is met when adverse effects on endocrine relevant endpoints in apical or supporting non-apical *in vivo* studies are supported by mechanistic data from *in vitro* or *in vivo* studies. Only when the sequence of the biochemical and cellular events that underlies the adverse effect is described and understood, can conclusive proof of endocrine disruption be considered as established. The other four scenarios (Scenarios A, B, D and E) describe data combinations from available studies that would result in the conclusion that there is no or insufficient evidence of endocrine disruption, and are discussed in Bars et al., 2011b.

Weight of the evidence and MoA

The principles of the WHO/IPCS conceptual framework for evaluating MoA for cancer and non-cancer endpoints (Boobis et al., 2006, 2008) should be applied for the weight-of-evidence evaluation of the available data. Briefly, the framework requires a description of the key toxicological events critical to the postulated MoA, followed by confirmation of a dose-response relationship, and a temporal association of the key events and the toxicological response. The strength, consistency and specificity of the effects then need to be determined, and the biological plausibility of the