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Workshop Report

Risk assessment of endocrine active chemicals: Identifying chemicals of regulatory concern

Remi Bars [a], Ivana Fegert [b], Melanie Gross [c], Dick Lewis [d], Lennart Weltje [e], Arnd Weyers [f], James R. Wheeler [d], Malyka Galay-Burgos [g,*]

[a] Bayer CropScience, 355 rue Dostoïevski, F-06903 Sophia Antipolis, France
[b] BASF SE, Carl-Bosch-Strasse 38, D-67056 Ludwigshafen, Germany
[c] WCA Environment Limited, Brunel House, Volunteer Way, Faringdon SN7 7YR, United Kingdom
[d] Syngenta Product Safety, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, United Kingdom
[e] BASF SE, Crop Protection – Ecotoxicology, Speyerer Strasse 2, D-67117 Limburgerhof, Germany
[f] Bayer CropScience, Building 6620, Environmental Safety – Ecotoxicology, D-40789 Monheim, Germany
[g] ECETOC, 4 Avenue E. Van Nieuwenhuyse, B-1160 Brussels, Belgium

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ABSTRACT

The European regulation on plant protection products (1107/2009) (EC, 2009a), the revisions to the biocides Directive (COM[2009]267) (EC, 2009b), and the regulation concerning chemicals (Regulation (EC) No. 1907/2006 'REACH') (EC.2006) only support the marketing and use of chemical products on the basis that they do not induce endocrine disruption in humans or wildlife species. In the absence of agreed 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 resulting ECETOC technical report (ECETOC, 2009a) and the associated workshop (ECETOC, 2009b) presented a science-based concept on how to identify endocrine activity and disrupting properties of chemicals for both human health and the environment. The synthesis of the technical report and the workshop report was published by the ECETOC task force (Bars et al., 2011a,b). Specific scientific criteria for the determination of endocrine activity and disrupting properties that integrate information from both regulatory (eco)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. However, to be able to discriminate chemicals with endocrine properties of low concern from those of higher concern (for regulatory purposes), the task force recognised that the concept needed further refinement. Following a discussion of the key factors at a second workshop of invited regulatory, academic and industry scientists (ECETOC, 2011), the task force developed further guidance, which is presented in this paper. For human health assessments these factors include the relevance to humans of the endocrine mechanism of toxicity, the specificity of the endocrine effects with respect to other potential toxic effects, the potency of the chemical to induce endocrine toxicity and consideration of exposure levels. For ecotoxicological assessments the key considerations include specificity and potency, but also extend to the consideration of population relevance and negligible exposure. It is intended that these complement and reinforce the approach originally described and previously published in this journal (Bars et al., 2011a,b).

1. Introduction

Recent European legislation (Plant Protection Products Regulation 1107/2009; proposed new Biocidal Products Regulation COM[2009]267) (EC, 2009a,b): 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. Substances with endocrine properties are also subject to authorisation under the European regulation on the Registration, Evaluation, Authorisation and Restriction of

Abbreviations: ACR, acute-to-chronic ratio; BfR, Bundesinstitut für Risikobewertung (German regulatory body); CLP, Classification, Labelling and Packaging; CRD, Chemicals Regulation Directorate (UK regulatory body); ECETOC, European Centre for Ecotoxicology and Toxicology of Chemicals; ED, endocrine disruptor; IPCS, International Programme on Chemical Safety; LOAEL, Lowest Observed Adverse Effect Level; MoA, mode of action; MoE, margin of exposure; NOAEL, No Observed Adverse Effect Level; NOEC, No Observed Effect Concentration; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; STOT-RE, Specific Target Organ Toxicity-Repeated Exposure; TDI, Tolerable Daily Intake; WHO, World Health Organisation.

* Corresponding author. Fax: +32 2 675 3625.
E-mail address: malyka.galay-burgos@ecetoc.org (M. Galay-Burgos).

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Chemicals (REACH; 1907/2006) (EC, 2006). Hence, the regulatory consequences of identifying a substance as an endocrine disrupting chemical are severe. However, the fundamental scientific criteria, necessary to define endocrine disrupting properties, are not described in any of these legislative texts or accompanying guidance.

Consequently, in response to these legislative developments and in absence of regulatory 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[1]). ECETOC presented this proposal at a workshop of regulatory, academic and industry scientists (Barcelona; June 29–30, 2009) to evaluate the approach as a concept for identifying endocrine disrupting properties within a regulatory context (ECETOC, 2009b[1]). The proposed guidance was refined following input from the workshop, and was published by Bars et al. (2011a,b). The proposed scientific criteria integrated, in a weight of evidence approach, information from regulatory (eco)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) of endocrine toxicity. Briefly, 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 or not (the reader is referred to Bars et al., 2011a,b for details). 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 (for regulatory purposes). However, the ECETOC task force recognised that this second part of the assessment needed further refinement.

A considerable amount of work has also been undertaken by individual EU member states and organisations, which has generated approaches for determining endocrine disrupting properties that have significantly progressed current thinking in this area (ECETOC, 2009a; BfR, 2011). Recognising this, the ECETOC task force hosted a second workshop (Florence; May 9–10, 2011), the aim of which was to evaluate the emerging guidance produced by regulatory authorities and organisations, to identify areas of concordance and difference, to consolidate the common scientific themes, and provide a platform for constructive debate on areas of potential difference. The outcome of that workshop has been published in a separate report (ECETOC, 2011). This paper presents the revisions made to the ECETOC guidance including some contributions from that workshop, and represents the view of the ECETOC task force. The focus of this paper is to elaborate on key aspects of the second part of the ECETOC guidance, and it therefore complements the approach previously published in this journal (Bars et al., 2011a,b).

2. Refinements to the ECETOC proposal to identify EDCs of regulatory concern for human health

The criteria proposed by the ECETOC task force (ECETOC, 2009a; Bars et al., 2011a,b) were based on two requisite elements shared by the broadly accepted definitions for endocrine disrupting chemicals (e.g. Weybridge, 1996; EC, 1999; IPCS, 2002; Japanese Ministry of the Environment, 2005), i.e. that exogenous substances need to cause adverse effects in intact organisms and that the adverse effect is caused by an endocrine MoA. In the development of the original guidance Bars et al. (2011) adopted the Weybridge definition. However, since the IPCS Bars et al., 2011a definition is currently the most widely accepted definition and also takes populations into account, the IPCS definition has now also been adopted in this revision to the guidance.

The current primary toxicology test methods for detecting endocrine toxicity in mammals are the standard regulatory OECD studies (e.g. 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 2–4 of the OECD conceptual framework for the testing and assessment of endocrine disrupting chemicals.

The first part of the ECETOC guidance considers five scenarios to guide the evaluation of available mammalian data to determine whether a substance has endocrine properties. Only one scenario (Scenario C; Bars et al., 2011) 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, (i.e. the sequence of the biochemical and cellular events that underlies the adverse effect is described and understood, then conclusive proof of endocrine disruption can 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. (2011).

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 MoA and effects are evaluated. The framework also suggests that other MoAs should be considered as a part of the overall weight of the evidence. If, after applying this framework to the evaluation of the available data, it is established that there is sufficient evidence to determine a substance as an endocrine disrupter, it is then necessary to discriminate chemicals of high regulatory concern from those of lower regulatory concern. This is an important consideration because not all substances, for which there is evidence of endocrine disruption, represent the same hazard to humans. Therefore, they should not all be of equal regulatory concern and subject to the same severe regulatory consequences, such as hazard-based exclusion under the pesticides legislation and authorisation under REACH. This can be illustrated with the example of caffeine, for which relevant adverse effects were observed in *in vivo* studies and are supported by *in vitro* mechanistic data. Decreased numbers of *copora lutea*, implantations and foetuses for F1 females were observed following dosing with caffeine in an apical rat reproduction study (Bradford et al., 1983). In supporting *in vivo* studies effects such as decreased sperm motility and increase sperm density were recorded in a mouse reproduction study (Gulati et al., 1984) and an increased incidence of resorptions was observed in rat developmental toxicity studies (Bertrand et al., 1965, 1970; Palm et al., 1978). In addition, an increased incidence of pituitary adenomas and mammary tumours were found in a 12 month rat chronic study (Yamagami et al., 1983) and in a 43 week mouse study (Welsch et al., 1988), respectively. Whilst no effects were found in an *in vitro* hER activation study, oestradiol

[1] ECETOC reports are available for free download at http://ecetoc.org/publications.