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WEIGHT OF EVIDENCE REVIEW TO DETERMINE ENDOCRINE DISRUPTING PROPERTIES OF ACTIVE SUBSTANCES

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INTRODUCTION

- The new European Regulation (EC) No. 1107/2009 on plant protection products (PPPs) sets out stricter criteria for approval of active substances.
- Active substances that are endocrine disruptors will not be approved under the new regulation unless there is negligible exposure to humans and non-target species.
- In addition to this, endocrine disrupting substances approved because of negligible exposure will become candidates for substitution with less hazardous substances during the authorisation stage at Member State level.
- However, the scientific criteria for defining an endocrine disruptor have not yet been defined in legislation. For PPPs the European Commission is to present draft measures concerning the scientific criteria by 14 December 2013. In the absence of regulatory criteria, various organisations and member states have put forward proposals for such criteria. These generally include the WHO/IPCS definition of an endocrine disruptor which combines evidence of an adverse effect in intact organisms with an endocrine mode of action. Several proposals also highlight the need for weight of evidence (WoE) considerations in the evaluation of the available data.
- We have reviewed two published WoE frameworks for the assessment of endocrine disrupting properties of chemicals (CEFIC EMSG 1999, Brown et al. 2001) and combined elements of each into a practical WoE evaluation for PPP active substances. The WoE evaluation takes into account and defines the differing levels of relevance provided by (eco)toxicological studies The WoE evaluation is a fundamental step in the assessment of any substance, and is compatible with the various proposals for criteria proposed to date.

METHODS

- The method makes full use of endpoints available in standard regulatory toxicity and ecotoxicity studies required for dossier submission for indications of endocrine effects (Table 1) and any relevant information in the open literature. Many of the apical regulatory studies fall into the higher tiers of the OECD Conceptual Framework for the testing and assessment of endocrine disrupters (OECD 2012), and therefore provide data on adverse effects on endocrine relevant endpoints.
- The combined framework consists of four evaluation steps:
  1. Study reliability — quality of work undertaken (using expanded Klimisch scores [Klimisch et al. 1997]).
  2. Study relevance — endpoint relevance to endocrine disruption (Table 2).
  3. Study significance — based on the earlier assessments made for reliability and relevance (Table 3).
  4. Balance of the weight of evidence, coherence and gap assessment. This step can be structured according to the OECD Conceptual Framework for testing and assessment of endocrine disrupters.

RESULTS

To date we have reviewed 17 active substances using this weight of evidence framework. Experience has shown that:

- Endpoints from regulatory toxicology and ecotoxicology studies provide relevant whole organism data for the assessment of endocrine disruption (effects on endocrine and reproductive organs, development, reproduction). These studies fall within Levels 4 and 5 of the OECD conceptual framework, which provide data on adverse effects on endocrine relevant endpoints.
- Regulatory studies mainly result in 'indicative' significance for the presence or absence of adverse effects relevant for the assessment of potential endocrine disruption, since endpoints in standard regulatory tests (currently required for dossier submission) may be influenced by disruption of the endocrine system, but are also known to be affected by other factors, e.g. environmental stress, systemic toxicity, etc.
- There is considerable variation in the amount of data available for different substances in the open literature, and the weight of evidence approach takes account of this.
- Expert judgement and interpretation of the available data are critical in the final overall assessment.

  An important consideration is the coherence of *in vivo* effects from long term studies and supporting mechanistic studies.
- If the balance of the weight of evidence indicates consistent evidence for adverse effects on endpoints relevant for the assessment of endocrine disruption, or is equivocal, targeted supporting *in vitro* and *in vivo* studies (either obtained from the open literature or commissioned by notifiers) are likely to be required to confirm an endocrine mode of action for any effects that are observed. The mechanistic studies required fall within Levels 2 and 3 of the OECD Conceptual Framework.
- If the balance of the weight of evidence strongly indicates that a substance does not cause adverse effects on endpoints relevant for the assessment of endocrine disruption, then further targeted supporting studies should not be required as there is no or limited evidence of adverse health effects in long term whole-organism tests (falling within Levels 4 and 5 of the OECD conceptual framework).

CONCLUSIONS

The dossiers required for the market authorisation of plant protection products provide comprehensive *in vivo* toxicology and ecotoxicology data packages. The available studies fall within Levels 4 and 5 of the OECD Conceptual Framework, and provide data on adverse effects on endocrine relevant endpoints. If the weight of evidence review indicates adverse effects on apical endpoints relevant for the assessment of endocrine disruption, or the weight of evidence is equivocal, targeted supporting *in vitro* or *in vivo* studies from Levels 2 or 3 of the OECD Conceptual Framework (either from the open literature or commissioned) are required to confirm an endocrine mode of action for any equivocal or adverse effects that are observed. If the weight of evidence review indicates that there are no adverse effects on apical endpoints relevant for the assessment of endocrine disruption, then further targeted supporting studies should not be required. This approach provides substantive, robust evidence for notifiers and regulators to prioritise substances in light of the new legislative requirements.

Table 1. Relevant toxicity tests required for 1107/2009 with examples of endpoints that may be related to endocrine disruption (modified from CEFIC EMSG 1999)

Tests required for 1107/2009 dossiers	Endpoints that may be related to ED
Mammalian tests	
Sub-acute/sub-chronic tests (rat and dog)	Weight and histopathology of gonads, reproductive tissues, and endocrine glands
Chronic/carcinogenicity tests (mice and rats)	Tumours and hyperplasia of gonads, reproductive tissues, and endocrine glands
Developmental tests (rats and rabbits)	• Reproductive tract malformations • Sex ratio • Spontaneous abortion/premature delivery • Embryo viability • Skeletal development
Multi-generation tests (rats)	• Developmental 'landmarks' • Weight and histopathology of gonads, reproductive tissues, endocrine glands • Impairment of reproductive performance • Nipple persistence in males • Anogenital distance • Sperm count, morphology and motility
Ecotoxicological tests	
One generation study in birds	Reproductive performance
Embryo-larval test in fish	Growth and development
Full-life cycle study in fish	Reproductive performance
Chronic toxicity in invertebrates	Reproductive performance

Table 2. Relevance of *in vitro* and *in vivo* assays and endpoints according to CEFIC EMSG

Relevance	Test type	Description
High	*In vitro*	• Endpoint is based upon receptor binding potential coupled with transcriptional activation in a whole cell or subcellular assay. • Receptor binding potential in a whole cell assay. • Assessment of steroid metabolism in a whole cell assay.
	In vivo	• Endpoint(s) in a multi-generational test or other repeat dose toxicity test that is specifically controlled by the endocrine system. • Parallel dose-response changes in hormone levels in the presence of consequent toxicological effects (mammalian only). • Negative data from a short term/screening assay specifically controlled by the endocrine system.
Medium	*In vitro*	• Endpoint is based on receptor binding activity in a subcellular assay. • Endpoint is based on cell growth or other endpoint, not a direct measurement of receptor mediated activity. • Endpoint of steroid metabolism in a subcellular assay.
	In vivo	• Endpoint in a multi-generation test, or other repeat dose standard toxicity test, which may be influenced by the endocrine system, but is also known to be affected by other factors, e.g., environmental stress, toxicity, etc. • Positive endpoint data from a short-term/screening assay specifically controlled by the endocrine system. • Changes in hormone levels in the absence of any toxicological changes (mammalian only).
Low	*In vitro*	Not applicable; all *in vitro* assays are relevant to at least some extent by definition.
	In vivo	Evidence indicates that the endpoint is not controlled by the endocrine system.

Table 3. Significance of *in vitro* and *in vivo* assays and endpoints according to CEFIC EMSG

Significance	Test type	Description
High[1]	*In vivo*[1]	• Chronic studies of high relevance and with reliability scores of 1 or 2.
Indicative	*In vitro*	• Studies of high relevance and with reliability scores of 1.
	In vivo	• Screening assay studies of high relevance and with reliability scores of 1 or 2. • Chronic studies of medium relevance and with reliability scores of 1 or 2.
Low	*In vitro*	• Studies of medium relevance and with reliability scores of 1, or high relevance and with reliability scores of 2.
	In vivo	• Screening assay studies of medium relevance and with reliability scores of 1 or 2.
Unusable	*In vitro/ in vivo*	• Data from studies with reliability scores of 3 or 4.

[1] The CEFIC EMSG framework does not allow for in vitro studies to be classified as High significance. At best these can only be "indicative" of mechanistic potential. However, a negative result of "Indicative" significance is sufficient to be definitive for the mechanism being investigated.

References: Brown et al. 2001. A critical review of the scientific literature on potential endocrine-mediated effects in fish and wildlife. Ecotoxicology and Environmental Safety 49:17-25; CEFIC EMSG. 1999. Towards the establishment of a weight of evidence approach to prioritising action in relation to endocrine disruption; Klimisch et al. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology 25: 1-5. OECD .2012. OECD 2012. Draft OECD Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption Available at: http://www.oecd.org/dataoecd/45/52/50459967.pdf [accessed 17/07/2012].

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