Risiken erkennen – Gesundheit schützen

Draft Concept Paper

Development of a Stepwise Procedure for the Assessment of Substances with Endocrine Disrupting Properties According to the Plant Protection Products Regulation (Reg. (EC) No 1107/2009)

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List of abbreviations

ARfD	Acute Reference Dose
BfR	Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)
CMR	Carcinogenic, Mutagenic, or Reprotoxic
CLP	Classifiaction, Labelling and Packaging
DIT	Developmental Immunotoxicity
DNT	Developmental Neurotoxicity
EC	European Community
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ED	Endocrine Disruption / Endocrine Disruptor
EDC	Endocrine Disrupting Chemical
EEC	European Economic Community
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EU	European Union
GHS	Globally Harmonised System
HPA	Hypothalamic-Pituitary-Adrenal
HPG	Hypothalamic-Pituitary-Gonadal
HPT	Hypothalamic-Pituitary-Thyroid
IPCS	International Programme on Chemical Safety
MoA	Mechanism or Mode of Action
MOE	Margin of Exposure
MRL	Maximum Residue Levels
NGO	Non-Governmental Organisation
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PPP	Plant Protection Product
RE	Repeated Exposure
REACH	Registration, Evaluation, Authorisation of Chemicals
SE	Single Exposure
STOT	Specific Target Organ Toxicity
TG	Testing Guideline
TTC	Threshold of Toxicological Concern
WHO	World Health Organization

1. Foreword

Endocrine disrupting properties of natural or synthetic chemical substances have become subject of scientific debate and public concern during the past decades, also due to observations of environmental and adverse health effects of such substances. Since in vertebrates the hormonal system is involved in regulating virtually all physiological processes, there are multiple organ systems, tissues and endpoints which may be affected by endocrine disruption (ED). Therefore, the assessment of endocrine disrupting properties is of great importance in regulatory toxicology.

In Europe, new legislation for the scientific evaluation and administrative regulation of chemical substances consequently addressing potential endocrine disrupting properties has entered into force or is currently in discussion.

According to the **new plant protection products Regulation** (EC) No 1107/2009, point 3.6.5. of Annex II, the Commission shall present a draft of the measures concerning specific scientific criteria for the assessment of endocrine disrupting properties to be adopted in accordance with the regulatory procedure by 14 December 2013. Therefore, the need for the development of a harmonised and scientifically based guidance document is obvious. In a first step, according to the draft guidance on Development and Use of Guidance Documents (SANCO/10581/2009 REV 0) a **concept paper** is presented as a draft proposal. This concept paper provides recommendations for the **development of a guidance document** on the assessment of active substances, safeners or synergists with potential endocrine disrupting properties in the context of Regulation (EC) No 1107/2009 concerning the placing of plant protection products (PPPs) on the market of the European Community.

Harmonised guidance is also considered necessary for **chemicals** addressed under Article 57(f) of Regulation (EC) No 1907/2006 as having endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health which give rise to an equivalent level of concern to those substances meeting the criteria for classification as carcinogenic, as mutagenic or as toxic for reproduction category 1A or 1B in accordance with Regulation EC No 1272/2008. Such substances should be included into the list of substances subject to authorisation (Annex XIV).

Based on the Proposal of the Commission for a Regulation of the European Parliament and of the Council concerning the placing on the market and use of biocidal products [COM(2009) 267 final from 12.6.2009] the same measures addressed under Article 57(f) of Regulation (EC) No 1907/2006 concerning specific scientific criteria for the assessment of endocrine disrupting properties should be applied as cut off criteria for the approval of **biocidal active substances** and their products.

As an important goal for the development of a new guidance on measures concerning specific scientific criteria for the assessment of substances with endocrine disrupting properties considering human relevance it is regarded necessary that they should be **applicable under the different areas of EC legislation** (chemicals under REACh as well as active substances in pesticides and biocides) taking into account specific aspects within the different areas, as appropriate.

As a result of an international workshop, held in Berlin 2009, a draft concept paper was provided by the BfR as a first input for the development of a harmonised European guidance document for use by Member State authorities and EC peer review groups and to assist Member States, EFSA and the European Commission when making decisions about the

approval of active substances, safeners or synergists with endocrine disrupting properties in accordance with point 3.6.5. of Annex II to Regulation (EC) No 1107/2009. It should also provide applicants with advice on the drafting of a dossier with a scientifically reasoned proposal for the assessment and classification of substances considered to have endocrine disrupting properties. This dossier should enable the Member States to evaluate an application for authorisation without the need to refer back to the applicant except for occasional clarification or further information, thus improving the efficiency and cost-effectiveness of the authorisation process.

2. Introduction

2.1. Legal background

Regulation (EC) No 1107/2009 concerning the placing of plant protection products (PPPs) on the market states in Article 4 (1) that the assessment of the active substance shall first establish whether the approval criteria set out in points 3.6.2 to 3.6.4 and 3.7 of Annex II are satisfied.
In the context of this concept paper, only the approval criteria set out in point 3.6 of Annex II (**impact on human health**) will be considered, whereas approval criteria relating to ecotoxicology (i.e. point 3.8.2. of Annex II; *endocrine disrupting properties that may cause adverse effects on non-target organisms*) are not taken into consideration.

The approval criteria which shall be taken into consideration first in the assessment of a substance (points 3.6.2 to 3.6.4 of Annex II) are as follows:

- "3.6.2. An active substance, safener or synergist shall only be approved if … it is not or has not to be classified … as mutagen category 1A or 1B."

- "3.6.3. An active substance, safener or synergist shall only be approved if … it is not or has not to be classified … as carcinogen category 1A or 1B, unless the exposure of humans … is negligible ..."

- "3.6.4. An active substance, safener or synergist shall only be approved if … it is not or has not to be classified … as toxic for reproduction category 1A or 1B, unless the exposure of humans … is negligible …"

If the approval criteria mentioned above are satisfied, then the approval criteria set out in point 3.6.5 of Annex II shall be considered:

- "3.6.5. An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with point (b) of Article 18(1) of Regulation (EC) No 396/2005.

By 14 December 2013, the Commission shall present to the Standing Committee on the Food Chain and Animal Health a draft of the measures concerning specific scientific criteria for the assessment of endocrine disrupting properties to be adopted in accordance with the regulatory procedure with scrutiny referred to in Article 79(4).

Pending the adoption of these criteria, substances that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties.

In addition, substances such as those that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties."

2.2. Definitions

It is proposed that, in the context of this concept paper for the development of specific scientific criteria for the assessment of substances with endocrine disrupting properties considering human relevance,

1. the (WHO/IPCS 2002) definition of the term "endocrine disruptor" should be used:

- **Endocrine Disruptor** (ED): "An ED is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations." (WHO/IPCS 2002)

2. the (WHO/IPCS 2004) definition of the term "adversity" (extended by the addition of the term "reproduction") should be used:

- **Adversity**: "A change in morphology, physiology, growth, [reproduction], development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences." (WHO/IPCS 2004)

3. suggestions for a draft definition for **negligible exposure** for further discussion are provided in annex V.

Further definitions needs to be discussed and harmonised (e.g. serious effects to human health) in the context of the development of guidance documents for **chemicals** and **active substances** in biocidal products addressed under Article 57(f) of Regulation (EC) No 1907/2006.

3. General considerations

The aim of this chapter is to provide a general reference to the stepwise approach of hazard assessment for an active substance, safener or synergist in PPP or biocidal products. Fundamental steps for scientific criteria in hazard assessment are based on the following principal steps:

- description of the toxicological profile of the substance;
- identification of the relevant critical effects in the most relevant and sensitive species;

- dose-response evaluation, i.e. identification of the LOAEL (lowest-observed-adverse-effect level) and the NOAEL (no-observed-adverse-effect level) for the critical effects;
- establishment of a mechanism/mode of action, if possible.

For the inclusion of an active substance in Annex I and for an application for the authorisation of a PPP, a comprehensive range of toxicological and toxicokinetic data must be assessed. The information for the chemical substance[1], taken together with that provided for one or more preparations containing the substance, must be sufficient to permit an evaluation to be made as to the risks for humans, associated with the handling and use of PPPs containing the active substance. These data requirements and test methods for the approval of a PPP are specified by Directive 94/79/EC amending Directive 91/414/EEC (see Table 1 in Annex IV).

It is recognised that endocrine modulation is not a hazard per se, but a mechanism/mode of action (MOA) of toxicity that may cause adverse effect in humans. Since all endocrine disruptors do not represent the same hazard to humans, a) an evaluation of the mechanism/mode of action (MOA) and its relevance for humans and b) an element or assessment of potency is also required to discriminate endocrine disruptors of high concern from those of lower concern.

Essentially, all mammalian toxicity and toxicokinetic studies required under Directive 91/414/EEC[2] (Annex IV) should be considered when assessing a chemical substance with endocrine disrupting properties potentially relevant to humans. Provided that other reliable relevant data are available, e. g. from peer-reviewed scientific literature, these should also be considered in the assessment process. In the case of suspected endocrine disruption, additional mechanistic data may be required to establish or support a certain mechanism/mode of action (MOA). A thorough investigation of the MOA for toxicity can help in the assessment of qualitative or quantitative differences between species.

In addition to the toxicity data chemical characterisation is regarded important to provide insights to key questions, including compounds stability, the potential for human exposure or the potential for accumulation.

Furthermore the OECD has developed a framework for testing and assessment of endocrine disrupting chemicals (OECD 2002). This approach, however, represents a toolbox and not a tiered approach for decision making (Annex IV). It is noted that highest tier tests are already included in the data requirements for plant protection products.

The required studies are expected to be able to provide evidence for endocrine effects. These include the recently amended short-term toxicity studies, the chronic toxicity/carcinogenicity studies, the (two-generation) reproduction studies, and the prenatal developmental toxicity studies. The latter studies are able to integrate the form and function of multiple biological processes, including those endpoints that are especially vulnerable to endocrine modulation. For the testing and assessment of potential endocrine disrupting chemicals, these so called apical *in vivo* assays (ECETOC 2009) can therefore provide the most relevant data about multiple endocrine mechanisms and effects i.e. they represent the current highest tier tests for detecting endocrine disrupting properties in mammals. It is a distinct

[1] In this paper an active substance, safener or synergist in a plant protection product

[2] Since the new European plant protection product regulation (Regulation (EC) No 1107/2009), replacing Directive 91/414/EEC, states that modified data requirements will be provided by June 2011, the old list remains valid until replaced.

advantage that studies of this type are usually available at the beginning of the hazard identification in cases of pesticide and biocide human health evaluation.

For certain chemical substances, there is some evidence that effects may not follow a monotonic dose response curve, potentially occurring at very low doses. Also, there is concern expressed by several scientists that endocrine disrupting effects might be overlooked in current guideline-conform toxicity testing. Therefore, triggered by evidence (provided that so-called low dose effects are further substantiated concerning robustness and reproducibility), improvement of testing methods with regard to the low dose range should be considered on a case-by-case basis, in order not to dismiss effects on the endocrine system which might be of relevance to human health.

4. Stepwise procedure for the assessment of substances with endocrine disrupting properties

For the assessment of substances with endocrine disrupting properties that may cause adverse health effects in humans, a **stepwise procedure** is proposed to support a science based regulatory decision process (Fig. 1). This stepwise approach (also referred to as 'tiered approach') is based on the recommendations of an international expert workshop, hosted by the BfR in Berlin 2009 (BfR 2010). In addition, it is proposed to establish criteria for classification of endocrine disruptors based on Regulation (EC) No. 1272/2008 (European Council 2008).

A stepwise approach is suggested that is based on specific scientific criteria established for substances with potential endocrine disrupting properties in plant protection products. These proposed criteria have been integrated into a decision tree (Annex 2). An important feature of the stepwise approach is the ability to exit the approach at any point when sufficient data have been evaluated for decision making. Within the proposed procedure science-based parameters such as adversity, biological or human relevance are used to analyze potential endocrine disrupting properties. For decision making in the final step of the tiered approach two options are suggested: An exposure-based option, taking into account (negligible) exposure and a classification-based option making use of classification and labelling according to criteria laid down under Regulation (EC) No. 1272/2008 and respective guidance documents.

It is recommended that the proposed measures should be tested upon a certain number of plant protection products to examine their applicability and to facilitate improvement of the framework.

In any case, the decision should be made on a case-by-case basis and must be based on expert judgement.

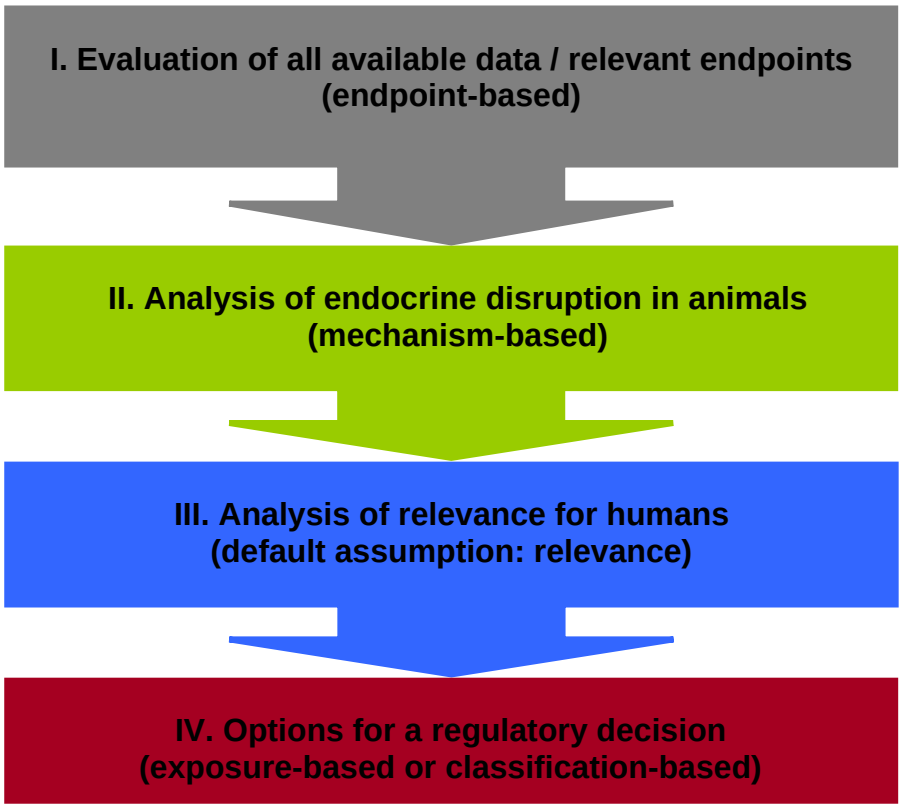

Figure 1: Conceptual framework for a tiered / stepwise approach for decision making. This tiered approach represents an overview and reflects the way a regulatory decision process may be made in general. A more detailed decision tree is shown in Annex 2. For the decision in step IV two options are suggested: Option 1 is exposure-based while option 2 is classification-based. A detailed description of these options is given in the text.

4.1. Step 1

In a first step, the proposal stipulates the chemical characterisation and an comprehensive analysis of all available toxicological data as a starting point for the decision process. These toxicity data required in a regulatory context as well as all other available data (including data from peer-reviewed scientific journals) should be analysed for potential hazards. Based on hazards identified by analysis of the respective toxicological endpoints, classification and labelling of the substance in accordance with Regulation (EC) No 1272/2008 (European Council 2008) can be proposed. Substances which would have to be labelled as carcinogenic, mutagenic or as reprotoxic (CMR) categories 1A and 1B would not be included into Annex I as requested by Regulation (EC) No. 1107/2009. Since the decision process on classification and labelling is not yet completed at this step, it is recommended to also include substances proposed to be labelled CMR 1A or 1B into the analysis of endocrine disrupting properties, and to clarify their mechanism of toxicity.

4.2. Step 2

In a second step, this endpoint-based analysis would be translated into a mechanism-based analysis. At this stage it is proposed to analyse all available data for effects / endpoints potentially caused by an endocrine disruptor, hence to analyse the potential endocrine mechanism(s) of the respective substance. Since hormonal regulation is involved in virtually all physiological processes of animals, it is crucially important at this stage to have criteria at hand to distinguish between physiological and adverse hormonal effects.

Several other potential criteria can be suggested for the evaluation of such harmful endocrine disrupting effects (Marx-Stoelting *et al.* 2009). Among these are adversity, specificity, dose-dependency as well as human relevance. Adversity in combination with toxicological relevance is considered the most important criterion for decision on ED for regulation at this stage. Adversity should be understood as defined above.

If there is no evidence for adverse / toxicologically relevant effects potentially related to ED, the decision tree can be left at this step. If on the other hand effects potentially related to an endocrine disruptive mechanism occur, which are regarded as adverse, it will be necessary to establish a mode of action in animals. One possibility for doing this might be the adoption of the IPCS conceptual framework, originally developed for evaluation animal carcinogenesis, for the assessment of the MOA of endocrine disrupting properties (Boobis et al 2006, 2008). For this purpose, additional mechanistic studies *in vivo* and *in vitro* may be necessary. The default assumption at this stage is that the mechanism is endocrine. If no mechanistic data are provided or if the mechanism of toxicity is shown to be endocrine, the substance may be considered as being an endocrine disruptor in animals. However, if the mechanistic data clearly show that the mechanism of toxicity is not based on endocrine effects, the substance is presumably not an endocrine disruptor and the decision tree can be left at this step.

4.3. Step 3

In a third step, relevance of effects observed in animal studies for humans will have to be analysed. The default assumption at this stage would be relevance. Consequently, only if a mechanism of toxicity in animals is identified that is clearly not relevant to humans, the decision tree might be left at this step.
If possible, detailed information on individual mechanisms of action should be established at this stage. Frameworks such as the IPCS frameworks for analysing the relevance of a cancer as well as a non-cancer mode of action for humans (Boobis et al. 2006, 2008) or other human relevance frameworks could be integrated into the decision process.

4.4. Step 4

The fourth step consists of the decision whether a substance would have to be regarded as an endocrine disruptor in a regulatory sense, i.e. whether it is considered to have endocrine disrupting properties that may cause adverse effect in humans or not. At this stage the final decision on approval or disapproval should be made, based on a critical assessment of exposure (option 1) or classification of the respective substance for ED properties (option 2).

As a more detailed elaboration of this stepwise approach a draft decision tree is presented in Figure 2 in the Annex.

4.4.1. Option 1

This exposure-based option foresees that an exposure analysis is performed to find out whether or not exposure to the respective substance for consumers as well as for operators, workers and others who might be exposed to the substance is negligible. This option emphasizes the generation and use of sufficient exposure data where possible for decision making and encourages the collection of appropriate exposure data. Approval of a substance with human relevant endocrine disrupting properties would only be possible if the exposure of humans under realistic proposed conditions of use is negligible as defined by Regulation (EC) No 1107/2009 in Annex II point 3.6.5.

A more science-based definition of negligible exposure is suggested in Annex V.

4.4.2. Option 2

This classification-based option for decision making suggests to amend criteria for classification and labelling and to introduce a classification system for endocrine disruptors for which there is scientific evidence of probable serious effects to human health which give rise to an equivalent level of concern to those substances meeting the criteria for classification as carcinogenic, as mutagenic or as toxic for reproduction category 1a or 1b in accordance with Reg. (EC) No. 1272/2008. A decision on approval or disapproval could then be based on the hazard identified and the classification applied for a respective substance. Endocrine disrupting properties would be treated like CMR-properties, for which a classification-based cut-off for substances classified as CMR 1A or 1B has been implemented in the new plant protection products regulation.

General considerations

It is proposed that for the assessment of substances which have endocrine disrupting properties in mammals, the principles for hazard classification and labelling as laid down in Regulation (EC) No 1272/2008 might be considered. However, this regulation does not provide specific criteria for the classification of potential health hazards of substances with endocrine disrupting properties in mammals. Therefore it is proposed to **adjust the basic classification criteria** appropriately taking into consideration the specific end-points which may be adversely affected by endocrine disruptors.

Substances with endocrine disrupting properties in mammals shall be classified as “endocrine disruptor (ED)” by the use of expert judgement, on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed. In this context it should be mentioned that for ED one effect may trigger more than one classification and labelling (e.g. C and / or R and ED).

Category 1

Substances are classified in Category 1 for “endocrine disruptor” on the basis of reliable and good quality evidence from human cases or epidemiological studies or observations from appropriate studies in experimental animals in which **severe toxic** effects on the endocrine system, assumed to be of relevance to human health, were produced at generally **low exposure** concentrations. Guidance on evaluation of effects and guidance dose values are based on those suggested for specific target organ toxicity after repeated exposure (STOT-RE) in Reg. (EC) No. 1272/2008 are provided in Annex III, to be used as part of a weight-of-evidence evaluation.

Category 2

Substances are classified in Category 2 for “endocrine disruptor (ED)” on the basis of observations from appropriate studies in experimental animals in which **significant toxic** effects on the endocrine system, assumed to be of relevance to human health, were produced at generally **moderate exposure** concentrations. Guidance on evaluation of effects and guidance dose values are based on those suggested for specific target organ toxicity after repeated exposure (STOT-RE) in Reg. (EC) No. 1272/2008 are provided in Annex III, to be used as part of a weight-of-evidence evaluation.

As a general rule, for the assessment and classification of substances with endocrine disrupting properties potentially relevant to humans, data from animal studies in mammals should have precedence over data from *in vivo* studies in non-mammalian species.

At present, data from most available *in vitro* test methods is not considered of sufficient weight of evidence on its own for regulatory decisions such as classification and labelling. However, such data may be helpful in the assessment of adverse effects on the endocrine system, for instance to clarify the mechanism/mode of action (MOA) and its relevance for humans. The quality of these studies and the adequacy of the data provided should be carefully evaluated on a case-by-case basis.

Approval of substances with endocrine disrupting properties based on the adjustment of the basic classification criteria according to CLP

It is proposed that an active substance, safener or synergist shall only be approved if, on the basis of assessment of endocrine disrupting toxicity testing carried out in accordance with the data requirements for active substances, safeners or synergists and other available data and information, including a review of the scientific literature, it is not or has not to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as **Endocrine Disruptor (ED) category 1**, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with point (b) of Article 18(1) of Regulation (EC) No 396/2005.

Substances that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as **Endocrine Disruptor (ED) category 2** shall not fall under the cut-off and go to regular risk assessment. Regular risk assessment is also foreseen to be conducted for substances with any combination of category 2 classifications for respective endpoints (like C, M or R cat. 2).

5. Summary

This concept paper for a guidance document provides a first draft based on the outcome of a BfR workshop[3] on how evaluation of endocrine effects of active substances, safeners or synergists to be used in plant protection products might be conducted. Its development has become necessary in the context of Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market of the European Community. Even though the criteria developed and the data set used as a basis for toxicological analysis of such substances are considered to be specific for plant protection products, it is recognized that a guidance document once developed might also have implications for other classes of substances such as biocides or chemicals under REACh. It is recommended that the proposed measures should be tested upon a certain number of plant protection products to examine their applicability and to facilitate improvement of the framework.

Concerning specific scientific criteria for the assessment of endocrine disrupting properties, a tiered approach for decision making is proposed reflecting single steps of the regulatory decision process on substances with endocrine disrupting properties for their use in plant protection products. In a first step all available data are evaluated in an endpoint-based manner. In a second step mechanisms of toxicity are analysed if present. Human relevance of these

[3] http://www.bfr.bund.de/cm/218/establishment_of_assessment_and_decision_criteria_in_human_health_risk_assessment_for_substances_with_endocrine_disrupting_properties.pdf

mechanisms of toxicity is examined in a third step. The fourth step consists of a decision. In this context two options are proposed which may be regarded as alternatives, representing to some extent complementary procedures:

Option 1: An **exposure-based approach** is suggested, that foresees exposure analysis and a decision based on the question whether or not exposure to an ED is negligible.

Option 2: In a **classification-based** manner it is alternatively proposed to adjust the current classification criteria laid down in Regulation (EC) No. 1272/2008 for substances with endocrine disrupting properties. As a next step, it is suggested to classify substances with endocrine disrupting properties based on the regulatory framework for specific target organ toxicity as endocrine disruptors (ED). While substances that have to be labelled ED category 1 would fall under the cut-off criterion, substances labelled ED 2 would not.

As a more detailed elaboration of this stepwise approach a draft decision tree is provided including specific criteria such as adversity or biological relevance. Definitions for ED and adversity used in this context are considered to be based on current WHO/IPCS recommendations.

Annex I: References

BfR. Workshop report. 2010. Accessible through http://www.bfr.bund.de/cm/218/establishment_of_assessment_and_decision_criteria_in_human_health_risk_assessment_for_substances_with_endocrine_disrupting_properties.pdf

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Annex II: Detailed draft decision tree

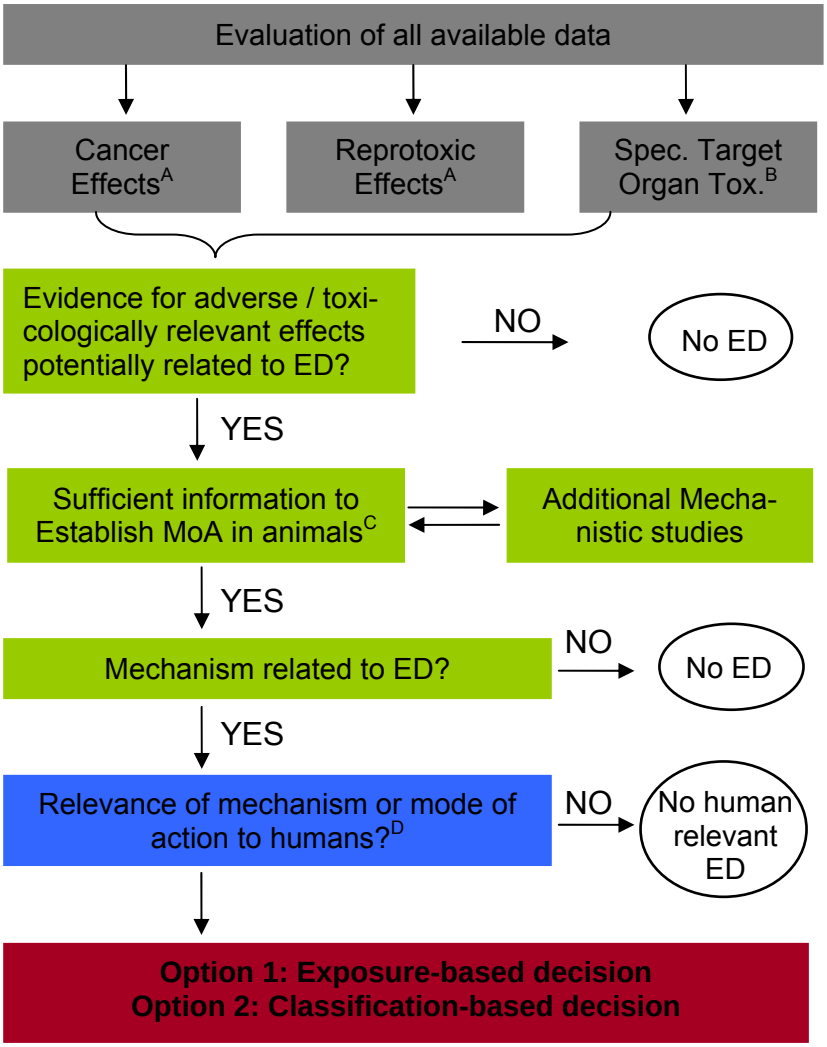

Figure 2: Detailed draft decision tree. Colours of boxes represent the relation to the four steps of the stepwise approach shown in Figure 1. After evaluation of all data an endpoint based hazard analysis is conducted (grey boxes). This is followed by an analysis of the mechanisms, which might have caused toxicity (green boxes). This mechanistic evaluation includes criteria like adversity as well as the establishment of a mode/mechanism of action in animals. If this mechanism is related to ED, its relevance to humans will be analysed with the default assumption being relevance to humans (blue box). After comparison to exposure a decision is made on ED in the last step (red box, option 1). Alternatively this decision might be based on classification (option 2).
A: Category 1A and 1B carcinogens and / or reprotoxicants are foreseen to be automatically banned at this stage. To clarify the mechanism of toxicity it is recommended to also analyse these substances for endocrine disrupting properties. B: Specific target organ toxicity (STOT) is a possible label for toxicity to any organ. In the context of endocrine disruption, not only effects on organs of the endocrine system, but also effects on the immune- or the nervous-system may be regarded as being of particular importance. C: Establishing of a mechanism or mode of action at this stage could consist of individual steps as suggested by the IPCS MOA framework. D: Relevance to humans could be assessed according to the IPCS MOA framework at this stage by analysing potential qualitative and quantitative relevance to humans separately.

Annex III: Further information on the proposed classification for ED

1. Effects considered to support classification for ED

For active substances, safeners or synergists used in plant protection products, the most relevant apical toxicology test methods for detecting substances which have endocrine disrupting properties in mammals are the short-term toxicity studies, the chronic toxicity/carcinogenicity studies, the (two-generation) reproduction studies, and the prenatal developmental toxicity studies.

Evaluation of data from these studies in experimental animals and of the available data from human experience/incidents is necessary to assess whether a consistent and significant toxic effect supports a classification for endocrine disrupting properties. Thus, all available evidence on the relevance to human health shall be taken into consideration in the assessment and classification process, including but not limited to the following toxic effects in humans and/or animals:

- significant functional changes in the endocrine system or hormone-dependent organs/tissues;
- any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters, only if related to endocrine disruption;
- significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination, only if related to endocrine disruption;
- morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction, only if related to endocrine disruption.

For the assessment of human relevance of a toxic effects observed in experimental animals, the IPCS framework for analyzing the relevance of a cancer as well as a non-cancer mode of action (MOA) for humans (Boobis et al. 2006, 2008) or any other feasible human relevance framework may be considered. The first step in this framework is to determine whether the weight of evidence based on experimental observations is sufficient to establish a hypothesized MOA. This comprises a series of key events causally related to the toxic effect. These events are then compared qualitatively and, next, quantitatively between experimental animals and humans.

2. Effects considered not to support classification for ED

It is recognised that effects may be seen in humans and/or in studies in experimental animals that do not justify classification. Such effects include, but are not limited to:

- clinical observations or small changes in bodyweight gain, food consumption or water intake that may have toxicological importance but that do not, by themselves, indicate significant toxicity;
- small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance;
- changes in organ weights alone with no evidence of organ dysfunction;
- adaptive responses that are not considered toxicologically relevant unless they lead to endocrine disruption;
- substance-induced species-specific mechanisms of toxicity, i.e. demonstrated with reasonable certainty to be not relevant for human health, shall not justify classification.

3. Guidance values to assist with classification for ED

In toxicity studies conducted in experimental animals, reliance on observation of effects alone, without reference to the duration of exposure and dose, violates a fundamental concept of toxicology, i.e. all substances are potentially toxic, and the toxicity is determined by the dose and the duration of exposure.

In order to help reach a decision about whether a substance shall be classified or not, and to what degree it shall be classified (Category 1 or Category 2), guidance values are provided for consideration of the dose which has been shown to produce significant toxic effects. The principal argument for proposing such guidance values is that all substances are potentially toxic and there has to be a reasonable dose above which a degree of toxic effect is acknowledged. It is therefore to be considered not only which effects have been produced, but also at which dose they occurred and how relevant they may be for humans.

Thus, when significant toxic effects are observed in animal studies that might warrant classification, the duration of exposure and the dose at which these effects were seen, in relation to the suggested guidance values, should be considered. Also, the decision to classify at all can be influenced by reference to the dose guidance values at or below which a significant toxic effect has been observed.

The guidance values proposed in Table 2 refer to significant toxic effects seen in a standard 90-day study. They can be used as a basis to extrapolate equivalent guidance values for toxicity studies of longer or less duration. The assessment shall be done on a case-by-case basis; for a 28-day study the guidance values are increased by a factor of three, and for a chronic toxicity study the guidance values are decreased by a factor of two. The values proposed in Table 2 are intended only for guidance purposes, i.e. to be used as part of the weight of evidence approach, and to assist with decisions about classification. They are not intended to be regarded as strict demarcation values.

Table 2. Guidance values for classification ED (in mg/kg bw per day).

Study type	ED 1	ED 2
28-day oral toxicity	≤ 30	≤ 300
90-day oral toxicity	≤ 10	≤ 100
Chronic toxicity	≤ 5	≤ 50

Annex IV: The OECD toolbox[4] for testing of ED and the list of mammalian toxicology studies required according to Annex II to Directive 91/414/EEC

Level 1 Sorting and prioritization based upon existing information	- Physical and chemical properties, e.g. MW, reactivity, volatility, biodegradability - Human and environmental exposure, e.g. production volume, release, use patterns - Hazard, e.g. available toxicological data	
Level 2 *In vitro* assays providing mechanistic data	- ER, AR, TR, receptor binding affinity - Transcriptional activation - Aromatase and steroidogenesis *in vitro* - Aryl hydrocarbon receptor recognition/binding - QSARs	- High throughout prescreens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate)
Level 3 *In vivo* assays providing data about single endocrine mechanisms and effects	- Uterotrophic assay (oestrogenic related) - Hershberger assay (androgenic related) - Non-receptor mediated hormone function - Others (e.g. thyroid)	- Fish VTG (vitellogenin) assay (oestrogenic related)
Level 4 *In vivo* assays providing data about multiple endocrine mechanisms and effects	- Enhanced OECD 407 (endpoints based on endocrine mechanisms) - Male and female pubertal assays - Adult intact male assay	- Fish gonadal histopathology assay - Frog metamorphosis assay
Level 5 *In vivo* assays providing data on effects from endocrine and other mechanisms	- 1-generation assay (TG415 enhanced)[1] - 2-generation assay (TG416 enhanced)[1] - Reproductive screening test (TG421 enhanced)[1] - Combined 28 day/reproduction screening test (TG422 enhanced)[1] [1] Potential enhancements will be considered by VMG mamm	- Partial and full life cycle assays in fish, birds, amphibians and invertebrates (developmental and reproduction)

[4] Adapted from http://www.oecd.org/document/58/0,3343,en_2649_34377_2348794_1_1_1_1,00.html

Table 1. List of mammalian toxicology studies required according to Annex II to Directive 91/414/EEC. The listed studies should be conducted in accordance with international guidelines such as OECD guidelines.

Annex Point	Area of toxicology	Specific studies
5.1./5.1.1.	Toxicokinetic studies	Absorption, distribution and excretion – following single and repeated oral administration
5.1.2.		Metabolism
5.2./5.2.1.	Acute toxicity	Oral
5.2.2.		Percutaneous
5.2.3.		Inhalation
5.2.4./5.2.5.		Skin and eye irritation
5.2.6.		Skin sensitisation
5.3./5.3.1.	***Short term toxicity***[5]	***Oral cumulative toxicity (28 day)***
5.3.2.		***Oral administration – two species, one rodent (preferably rat) and one non-rodent, usually 90-day study***
5.3.3.		Other routes if appropriate
5.4.	Mutagenicity	Test battery to assess gene mutations, chromosomal aberrations and DNA perturbations
5.5.	***Long term toxicity and carcinogenicity***	***Oral long term toxicity and carcino-genicity (rat and other mammalian species) – other routes as appropriate***
5.6./5.6.1.	***Reproductive toxicity***	***Multi-generation studies***
5.6.2.		**Developmental toxicity studies**
5.7./5.7.1	Neurotoxicity studies	Neurotoxicity studies in rodents
5.7.2.		Delayed polyneuropathy studies
5.8./5.8.1.	Other toxicological studies[6]	Toxicity studies of metabolites as referred to in the introduction
5.8.2.		Supplementary studies on the active substance
5.9./5.9.1.-5.9.8.	Medical data	Medical surveillance on manufacturing plant personnel; direct observations (e.g. clinical cases and poisoning incidences); health records from industry and agriculture; epidemiological studies; diagnosis of poisoning; allergenicity observations; proposed treatment and prognosis of expected effects of poisoning
5.10	Summary of mammalian toxicity and overall evaluation	

[5] Target organs, where relevant (especially immune, nervous and endocrine systems; if nervous system, immune system or endocrine system are *specific* targets in short term studies at dose levels not producing marked toxicity, additional second tier tests including functional testing should be considered).

[6] In certain cases it can be necessary to carry out supplementary studies to clarify observed effects further. These studies could include studies on potential effects on the endocrine system.

Annex V: Negligible exposure

- Exposure of **„workers, consumers and bystanders“** to the active substance of a biocidal product is considered negligible with respect to ED if
  a.) total systemic exposure or local exposure counts for less than 10% of the corresponding reference value (AEL) or
  b.) the active substance is not genotoxic and the total internal exposure to the active substance does not exceed 1.5 µg per person and day.

  Explanation
  a.) The definition of negligible exposure as percentage of a reference value is the most transparent and verifiable option. A general margin of 1000 to the lowest NOAEL (assuming a general assessment factor of 100) is considered safe. The percentage value of 10% is sufficiently low to leave room for any additional exposure from unknown sources. This definition also prevents the inclusion of substances for which a threshold value cannot be set (e.g. most genotoxic carcinogens).
  b.) The value of 1.5 µg/person/d is derived from a statistical evaluation of chronic studies by Munro et al. (1996) for more than 600 substances. Below this amount the risk to human health from any substance (except genotoxic carcinogens) is considered negligible. If this criterion should be applied, it is considered necessary to prove by appropriate tests, preferably in vitro, that the substance is not genotoxic.