Risiken erkennen – Gesundheit schützen

Establishment of assessment and decision criteria in human health risk assessment for substances with endocrine disrupting properties under the EU plant protection product regulation

Report of a Workshop hosted at
the German Federal Institute for Risk Assessment (BfR) in Berlin, Germany,
from Nov. 11th till Nov. 13th 2009

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Note: This meeting report reflects the discussion and results of the workshop. It does not necessarily or entirely detail the opinion of every single participant or the opinions of the institutions or companies they work for.

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1. Abstract

Chemical substances with a potential to modulate the hormonal system may have harmful effects on human or animal health, if they are included in plant protection products. Consequently, the new EU plant protection regulation (EC) No 1107/2009 names as one of the cut-off criteria that an active substance, safener or synergist shall only be approved if it is not considered to have endocrine disrupting properties that may cause adverse effects in humans, unless the exposure of humans under realistic proposed conditions of use is negligible. However, the new regulation fails to provide measures concerning specific scientific criteria for the assessment and decision on substances with endocrine disrupting properties. Specific criteria are to be presented by the European Commission within four years.

To address this need, the German Federal Institute for Risk Assessment (BfR) hosted an expert workshop to establish assessment and decision criteria in human health risk assessment for substances with potential endocrine disrupting properties. It was strongly recommended by a majority of workshop participants to replace the preliminary interim criteria implemented in the regulation (EC) No 1107/2009 in the decision making process by specific scientific criteria at the earliest time possible. Prior to the workshop, a conceptual framework for evaluating potentially adverse endocrine effects and their relevance for humans under realistically proposed exposure conditions was presented by the BfR. Central aspects considered and discussed within this conceptual framework for a tiered decision process included the analysis of adversity of effects on the endocrine system, of mechanistic data to establish a mode or mechanism of action in animals, and of relevance of such effects to humans. The proposal was modified and considered useful as a starting point for the development of measures to be adopted in accordance with the regulatory procedure.

In addition, the importance for extending dosing in toxicity studies to the low dose range and to acknowledge critical windows of development as well as a potential need to refine current testing guidelines and study designs were critically discussed and the necessity for research and further international projects on these issues was emphasised. The recommendations of the workshop and proposals for next steps are to be presented to the European Commission and EFSA.

2. Introduction

Chemical substances with a potential to modulate the hormonal system may be expected to have harmful effects on human or animal health, if they are included in plant protection products. Since such endocrine disrupting chemicals have been subject to intensive scientific investigation and discussion (for recent overviews see Diamanti-Kandarakis *et al.* 2009; Shaw 2009), chemical substances with endocrine disrupting properties have recently been specifically addressed in the new European legislation for pesticides, biocides and for other chemicals under REACH, respectively (for overviews on regulation see Beronius *et al.* 2009; Harvey and Everett 2006).

The new European plant protection products regulation was published on November 24th as Regulation (EC) No 1107/2009 and the legislation will fully enter into force in June 2011. Among other approval criteria, Regulation (EC) No 1107/2009 names endocrine disrupting (ED)[1] properties as a cut-off criterion for the approval of substances for use in plant protection products (European Council 2009). However, the regulation fails to provide specific scientific criteria for the evaluation of substances with potential endocrine disrupting properties. Such criteria are to be presented by the Commission within 4 years (by 14 December 2013).

To address the need to develop specific scientific criteria, the German Federal Institute for Risk Assessment (BfR) has proposed a draft concept involving a regulatory decision tree, and hosted a workshop to discuss these scientific criteria for the assessment of substances displaying endocrine disrupting properties with potential to induce adverse effects on human health. The workshop took place from November 11th to November 13th at the premises of the Federal Institute for Risk Assessment in Berlin-Marienfelde, Germany. Fifty-three experts from 16 countries / international bodies and with different institutional backgrounds (academia, regulatory bodies, industry and NGO) participated in the meeting. A list of participants is shown in Annex II.

Aims of the workshop were to discuss and develop scientific criteria for the regulatory decision on whether or not substances may be regarded as having endocrine disrupting properties that may cause adverse health effects in humans, to provide a draft conceptual framework for cut-off criteria on approval or disapproval of substances for their use in plant protection products, and to propose recommendations and next steps on the improvement and endorsement of this decision framework.

[1] A list of all abbreviations used in this report is provided in Annex VI.

Thus, within the context of development of decision criteria on endocrine disruption, the workshop was focussed on regulatory human health risk assessment and on regulation of active substances in plant protection products rather than on other areas of chemical regulation. It is, however, foreseen that criteria once set for one area of regulation might also have implications for other classes of substances, e. g. for biocides or chemicals regulated under REACH.

A pre-workshop survey was conducted to identify issues associated with a high level of agreement among participants as well as issues of controversy, and to structure and facilitate discussion at the workshop accordingly.

The workshop programme (see Annex I for details) comprised a series of presentations on the first day followed by group work in breakout groups on the second day. Results of the breakout groups were presented at the end of the second day. The workshop was concluded by a plenary discussion on the third day.

Introductory presentations summarised the results of the pre-workshop survey and introduced a draft conceptual framework suggested by the BfR. The subsequent presentations focussed on the issue of endocrine disruption within the new plant protection products regulation and related regulations such as REACH, on the informative value of studies and methods for analysing potential hazards caused by endocrine disruption, on potential targets and mechanisms of endocrine disruption, on the importance of testing the appropriate dose range and timing in studies assessing endocrine disrupting chemicals, and on assessment of human relevance of endocrine disruption.

Topics of the breakout groups were: Informative value of studies and methods (group I), targets and mechanisms of endocrine disruption (group II), dose relevance and criteria for adverse effects in animal studies (group III), and human relevance of evidence for endocrine disruption (group IV).
Each group was asked to discuss up to three questions selected from the questionnaire and potentially arising related questions. In addition, toxicological data on an example active substance of a plant protection product was to be analysed to test the practicability of the proposed criteria and decision tree. Furthermore, each group was asked to summarise its results and to present future recommendations. Summaries were presented in a plenary session on day two. An overview of the results of all breakout groups was presented and extensively discussed by the plenum on day three.

The following meeting report summarises the results of the breakout groups and the plenary discussions at the workshop. It is intended to serve as a protocol integrating different opinions rather than as a consensus statement.

3. Workshop results

3.1. Results of a pre-workshop questionnaire

Prior to the workshop, a questionnaire was sent out to confirmed participants to obtain their input at an early stage, identify pertinent issues, and to prepare and structure the discussion in the workshop breakout groups. Based on the questionnaire evaluation, many of the questions were scheduled for discussion in breakout groups. Detailed results of the questionnaire are presented in Annex III.

3.2. Results of the breakout groups

The four breakout groups discussed different questions identified by the pre-workshop questionnaire, and also related questions if there was time (for detailed results see Annex III). In addition, the suggested decision tree was tested for applicability with four different examples of active substances in plant protection products. Breakout groups summarised their results, and subsequently, the results of the individual breakout groups were presented and discussed in a first plenary session on November 12th. An overall summary of the results of the breakout groups was discussed in the final plenary session on November 13th. Results of both plenary sessions are presented below. Detailed results from the breakout groups are presented in Annex IV.

3.3. Results of the plenary sessions

Major points of discussion related to studies and methods, assessment and decision criteria, assessment of human relevance, low dose exposure and timing of exposure, definitions of negligible exposure and issues of classification and labelling. The discussion on these subjects is summarised below under different headings:

3.3.1. Definitions

Definitions for endocrine disruption and adversity, which would be feasible in a regulatory context, were discussed. A high level of agreement was obtained for implementation of the following definitions for regulating plant protection products in the European Union:

- **Endocrine Disruptor:** The WHO/IPCS definition was preferred and also used as a working definition for ED during the workshop:
  "An endocrine disruptor (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]

- **Adversity:** After controversial discussion in breakout group III, the WHO/IPCS definition (extended by the addition of 'reproduction') was suggested as a working definition for 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]

3.3.2. Studies and methods

The intense scientific investigation and discussion of studies and methodology for detection of endocrine disrupting effects of substances is reflected by numerous scientific publications and overviews (e.g. Baker 2001; Clode 2006; Naciff and Daston 2004) as well as by recommendations of methodological frameworks by international bodies such as the OECD (OECD 2002). Additionally, internationally harmonised guidelines have been revised (or are currently under revision) for enabling better detection of potential endocrine disruptive toxicity (Gelbke *et al.* 2004; Gelbke *et al.* 2007; Moon *et al.* 2009; Owens *et al.* 2007; Owens and Koeter 2003).

At the workshop the discussion on studies and methods had a dual focus. On the one hand it was asked whether the core studies laid down in the plant protection products legislation, which need to be submitted by an applicant for the approval of an active substance for its use in a plant protection product, are sufficient to provide evidence for endocrine adverse effects. An overview on studies as required by Directive 91/414/EEC (European Council 1991) is given in Table 1.[4]

2 It is noted that this definition was not agreed upon by all participants. A minority of attendants felt that there was no need for the definition to contain the term "adverse", that endocrine disruption should be defined on the basis of what happens in the human body resulting from alterations involving endocrine systems, and that any endocrine modulation might have the potential to lead to perturbance of human health.
3 It was stated that some aspects of the definition may be difficult to implement or to apply with the current testing guidelines. However, as a working definition, it was regarded feasible.
4 Directive 91/414/EEC is to be replaced by the new European plant protection products legislation, Regulation (EC) No 1107/2009. Since this new regulation states that modified data requirements will be provided by June 2011, the old list remains valid until replaced.

Table 1: List of studies required according to Annex II of the EU plant protection product directive 91/414/EEC. The listed studies should be conducted in accordance with international guidelines such as OECD guidelines.

Annex II Point	Area of toxicology	Specific studies
5.1./5.1.1.	Metabolism studies in mammals	Absorption, distribution and excretion – following both oral and percutaneous administration
5.1.2.		Elucidation of metabolic pathways
5.2./5.2.1.	Acute toxicity	Oral
5.2.2.		Percutaneous
5.2.3.		Inhalation
5.2.5.		Skin and - where appropriate - 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 carcinogenicity (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
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	

A second focus was on the design of the individual endpoint studies as laid down in respective internationally harmonised guidelines such as OECD-guidelines. Here the question was raised whether these testing protocols (e.g. for short-term toxicity, carcinogenicity, developmental toxicity or reproductive toxicity) are comprehensive enough to detect all adverse effects which might be caused by endocrine disruption in mammalian test organisms. The recommendations of the workshop are presented below. For a more detailed discussion see results of the breakout groups (breakout group I) in Annex IV.

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

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

- It was recommended to consider all relevant endocrine mechanisms and systems and to integrate mechanism-based analysis.
  (This is, however, already implemented in the directive, as mechanistic studies to clarify a mechanism of toxicity may be required).
- The core data set as laid down in Directive 91/414/EEC was considered to provide some evidence on potential adverse effects, but it was stated that it is not clear, if all possible aspects of ED would be captured.
- There were concerns that the existing study designs might not be comprehensive enough to detect all adverse effects which might be caused by endocrine disruption in mammalian test organisms.
  In this context, the discussion focussed on potential effects in the low dose range (cf. 3.3.4) and on critical time windows in development. There was concern that, because animals may not be evaluated for relevant endpoints during all life stages, certain endpoints such as developmental neurotoxicity (DNT) or developmental immunotoxicity (DIT) might be missed. Several participants brought forward a recommendation that information from extended one-generation studies including DNT and DIT modules should be considered for the decision process, if reliable data are available.
- There was also a discussion on the need for additional endpoints to assess all parts of the endocrine system.

3.3.3. Assessment and decision criteria

Preliminary criteria for the decision on ED are laid down in Reg. (EC) No. 1107/2009. These criteria suggest that 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 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.

These preliminary criteria laid down in Reg. (EC) No. 1107/2009 were criticised as not justified from a scientific point of view and it was strongly recommended that specific scientific criteria should be developed to replace the interim criteria as soon as possible. Therefore, additional criteria were suggested to be integrated into a decision process on potential endocrine disruptors. Among these the following were discussed: Adversity,

specificity, dose dependency, biological and human relevance. The discussion took place mainly in the breakout groups (see Annex IV for more details). Some central aspects are however summarised below:

Discussion on adversity

- Adversity was favoured as a criterion for assessment and decision of substances with endocrine disrupting properties under the new EU plant protection products legislation.
- Since adversity is a central part of several definitions of ED (e.g. Weybridge 1996 or WHO/IPCS 2004) it is important to integrate this into a decision process on ED.
- It was recommended to connect adversity to toxicological relevance.
- The definition of WHO/IPCS 2004 was suggested for adversity, extended by the addition of 'reproduction'.

Discussion on specificity

There was a controversial discussion on this criterion, and different opinions were brought forward:

- Effects on the endocrine system or its target organs seen at dose levels causing marked generalised toxicity are likely to be the non-specific secondary consequence of the marked generalised toxicity and should not be regarded as specifically endocrine disruptive. Substances showing those effects should be subject to regular risk assessment under the EU PPP Regulation.
- If endocrine adverse effects occur only above the dose at which a different form of toxicity occurs, the apparently more sensitive effect (due to other mechanisms of toxicity) could be used for regulatory purposes (derivations of reference values), so the endocrine effect could be assumed to be covered by regulation.

  The opinions above were strongly opposed by some participants. In particular, concern was expressed that critical effects resulting from endocrine disruption, but concerning the developing organism, might be regarded as not relevant for the decision process if they occurred at dose levels above those causing maternal toxicity.
- It was the opinion of many participants that an effect should not be regarded as being non-specific or not relevant purely on the basis of being secondary (the result of a primary effect). E. g. liver enzyme induction may lead to a decrease in circulating hormone levels, which may in turn have an impact on target organs. Accordingly, these attendants advised regulators to consider all effects related to endocrine

disruption for the decision process, regardless of whether they may be deemed to be primary or secondary.

- A related statement implied that endocrine disruption should be defined via the mechanistic data rather than on the decision of whether an effect is regarded as primary or secondary: Among others, one central issue of endocrine disruption is generally whether the effect is ultimately a receptor-mediated event. If there is evidence that endocrine disruption is the critical effect based on mechanistic studies, then effects should be regarded as relevant for the decision process.

Discussion on relevance to humans

One of the aspects in the decision process on endocrine disrupters involves the extrapolation from animal data to the human situation. The BfR proposal initially presented to the participants at the beginning of the workshop suggested to implement the human relevance framework developed by the IPCS for cancer and non-cancer mode of action within the decision process on endocrine disruptors (Boobis *et al.* 2006; Boobis *et al.* 2008). This framework was critically analysed in detail (Guyton *et al.* 2008), but has been claimed to be useful in a regulatory context (Meek *et al.* 2008). During the workshop, the IPCS human relevance framework was controversially discussed and it was recommended to focus on mechanisms of action (in terms of individual mechanistic steps rendering a more solid basis of scientific understanding) rather than on a mode of action (which is regarded as representing only a more general understanding) in the assessment of human relevance. In this context “mechanism of action” should be defined as the totality of mechanistic steps necessary for a certain toxic effect while “mode of action” comprises a less detailed description of the mechanism or several key events within the mechanism (Guyton *et al.* 2008). If, however, it would not be possible to establish a complete mechanism of action, mode of action analysis would still be feasible (for a more detailed discussion see below and results of breakout group IV):

- The default assumption is that effects noted in animals are relevant to humans; it can be rebutted with additional (mechanistic) data.
- Mechanism of action is the recommended context for determining relevance.
- The current regulatory system is based on endpoints. Endpoints observed in toxicity studies should be translated into mechanisms where possible.

3.3.4. Low dose effects

One major point of a controversial discussion in all breakout groups was the question whether effects may occur at low doses (e. g. at realistic human exposure levels), below those traditionally tested. Low dose effects were also discussed in some of the introductory

lectures. Since concern was raised that effects due to non-monotonic dose-response curves might be overlooked in current guideline-conform toxicity testing for which doses are employed that typically exceed human exposure by orders of magnitude (Beronius et al., 2009), it was regarded as important to discuss and clarify this issue. In the course of the discussion the following points were mentioned / suggested by a number of experts:

- Part of the participants raised concern that, based on current data sets, endocrine effects might be missed for certain compounds in the lower dose range.
- It was recommended, triggered by evidence, to encourage improvement of testing in respect to the low dose range.
- However, concerns on the robustness of low dose effects were also addressed by other participants.
- Reproducibility of effects and validation would be required for the inclusion of low doses in testing guidelines (amendment of existing guidelines). The question on how to design a low dose test was raised and the importance of validation emphasised.
- The need for concrete examples for substances and/or endpoints that are not currently captured by the typical range of dose levels used in guideline-testing, to decide which endpoints and low dose levels should be included into existing guidelines was mentioned by some participants.
- It was critically mentioned by other participants, that increasing the minimum number of dose levels would require more animal testing. In this context it was noted that guidelines usually do not dictate dose levels but recommend an appropriate number of doses (minimum three) and that the highest dose should induce toxicity.
- To examine the potential for adverse effects at a low dose level (below a “traditional” NOAEL), it was further recommended to develop, validate and apply “new” methodology (e. g. array approaches) aiming at identification and interpretation of additional mechanistic (receptor-mediated) effects. Clarification on how to do this in practice was also recommended.
- To bring the low dose issue forward in a productive way, several recommendations were made:
    - Robust evidence of low dose effects of endocrine disrupting substances was considered to be important to be established before regulatory action might be taken. This evidence should include reproducibility of effects with the same compound in different studies.
    - Funding of international projects for the validation of methods and development of new methodology and
    - funding of literature search on evidence for potential low dose effects of substances with endocrine disrupting properties were recommended.

- The necessity of workshops on low dose issues was considered to be of major relevance.

3.3.5. Negligible exposure

The regulation (EC) No. 1107/2009 states that a substance with endocrine disrupting properties should not be approved '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' (European Council 2009). It states further, that negligible means that the product would be used in closed systems or in other conditions excluding contact with humans and that residues of the active substance, safener or synergist concerned in 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 (European Council 2005), which is 0.01 mg/kg food. At the workshop the definition of negligible exposure as laid down in the regulation was critically discussed. Many participants disagreed with the setting of a default value, as this would not account for different potencies of different substances and because a default value might not adequately cover substances which have critical effects below this value. As science-based concepts, the margin of exposure (MOE) or the threshold of toxicological concern (TTC) were suggested. Both concepts have been discussed for carcinogenic substances in food (Barlow and Schlatter 2009; Pratt *et al.* 2009). The TTC concept is presented in more detail by Kroes *et al.* (2005). Critical points discussed at the workshop were:

- A maximum residue level (MRL) of 0.01 mg/kg food for all compounds is not a health-based scientific decision criterion to protect consumers.
- Closed systems do not necessarily exclude exposure of bystanders and residents.
- It was strongly recommended that a science-based definition of negligible exposure should be preferred to a default value.

3.3.6. Classification and labelling

Classification and labelling in the European Union is performed according to Regulation (EC) No 1272/2008. It was discussed, whether classification and labelling of ED should be performed according to this regulation or whether it would be advisable to develop a new system for endocrine disruptors. The following suggestions were made:

- At first the adopted regulatory system for classification and labelling should be applied.
- In the next phase it was regarded by some participants to be necessary to develop a classification system for endocrine disrupting chemicals considering issues such as adversity etc.

- It was discussed whether a system similar to that applied for mutagenicity, carcinogens or reproductive toxicity, or rather a yes/no classification, or a system based on level of concern should be recommended.
- A critical point was the suitability of interim criteria.
  - Preliminary criteria as laid down in Reg. (EC) No. 1107/2009 (Carcinogenic Cat. 2/Reprotoxic Cat. 2 substances are to be regarded as EDs) were considered as not science-based.
  - The necessity was emphasised to develop specific scientific assessment criteria to replace the preliminary interim criteria.

3.4. The BfR tiered approach and decision tree

A tiered approach was suggested by the BfR, intended to reflect a possible decision process for substances with potential endocrine disrupting properties in plant protection products (original version shown in Annex V). As a more detailed elaboration of this tiered approach a draft decision tree was presented including specific science-based criteria such as adversity. The workshop discussions have provided recommendations for an improvement of this framework. The tiered approach and the decision tree were considered applicable for the examples discussed at the workshop and useful as a starting point for the development of measures concerning specific scientific criteria for the determination of endocrine disrupting properties to be adopted in accordance with the regulatory procedure by the participants of the workshop. The modified approach is described in the following (Figure 1 giving an overview on the tiered approach and Figure 2 showing a detailed draft decision tree. Colours in Figure 2 represent single steps of the tiered approach). In any case, it was recommended that the decision should be made on a case-by-case basis and should be based on expert judgement.

Step I

In a first step, the proposal stipulates the analysis of the toxicological data provided by the applicant as required in Annex II of Directive 91/414/EEC (see Table 1 for a list of all studies required) as a starting point for the decision process. These Annex II toxicity data as well as all other available data (including data from peer reviewed scientific journals) would be analysed for potential hazards (e.g. carcinogenicity, reproductive or developmental toxicity) by the responsible authority. Based on hazards identified by analysis of the respective toxicological endpoints, regulatory agencies would suggest classification and labelling of the substance in accordance with Regulation (EC) No 1272/2008 (European Council 2008).[7]

[7] It should be noted at this stage that classification and labelling for regulatory purposes has to be made in accordance with current legislation and that regulators are legally bound to this legislation.

Substances which would have to be labelled as carcinogenic, mutagenic or as reprotoxic (CMR) categories 1A and 1B in accordance with Regulation (EC) No. 1272/2008 would be excluded from Annex I (banned) as requested by Regulation (EC) No. 1107/2009 at this step.[8] Since the decision process on classification and labelling regarding CMR may not yet be completed, it was recommended during the workshop 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. If a substance proposed to be labelled CMR 1A or 1B were, in addition, an endocrine disruptor, it would be considered as especially noxious (severe).

Step II

In a second step this endpoint-based analysis would be translated into a mechanism-based analysis to the extent possible as recommended during the workshop. At this stage it would be required to regard all effects / endpoints separately, and to analyse all available data for effects potentially caused by an endocrine active substance, 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.

The BfR had suggested several potential criteria for the identification of such harmful endocrine disrupting effects (Marx-Stoelting *et al.* 2009). Among these were adversity, specificity, dose-dependency as well as human relevance. These criteria were all integrated into the original draft decision tree (since a modified version of this decision tree was prepared to integrate the results and recommendations of the workshop, the first draft is only shown in Figures 3 and 4 of Annex V, while the modified versions are presented in Figures 1 and 2 in the main document). Since discussion on specificity and dose-dependency was controversial, adversity (in combination with toxicological relevance as suggested during the workshop) remains the most important criterion for decision on ED for regulation at this stage. Adversity should be understood as recommended by the majority of workshop participants and as defined by WHO/IPCS 2004.

If there is no evidence for adverse / toxicological 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 (or better: a mechanism) of action in animals. For this purpose, additional mechanistic studies *in vivo* and *in vitro* may be required. The default assumption

[8] For carcinogenic and reprotoxic substances, Reg. (EC) 1107/2009 also requests comparision to exposure and allows approval of a substance if exposure is negligible as defined in point 3.6.3 and 3.6.4 of annex II of the regulation.

at this stage is that the mechanism is endocrine. If no mechanistic data are provided or if the mechanism of action is shown to be endocrine, the substance may be considered being an endocrine disruptor in animals. However, if the mechanistic data clearly show that the mechanism of action is not based on endocrine effects, the substance is presumably not an endocrine disruptor and the decision tree can be left at this step.

Step III

In a third step, relevance of effects observed in animal studies for humans will have to be analysed. Some participants mentioned that the default assumption at this stage has to be that animal findings are relevant to humans.[9] 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.

It was originally suggested to implement the IPCS mode of action framework (Boobis et al. 2006, 2008) at this step. This was controversially discussed, as there was disagreement among some participants on the applicability of this framework (see discussion on human relevance). As a consequence, the IPCS framework was not integrated into the proposed decision tree, but it was left to the decision of the regulators performing human relevance analysis, which human relevance framework should be used. In this context regulators might however be bound to regulatory frameworks which are generally accepted by international regulatory bodies.

[9] It should however, be noted that regulatory procedures in the European Union and other international Regulatory Authorities have usually considered human relevance as a default assumption.

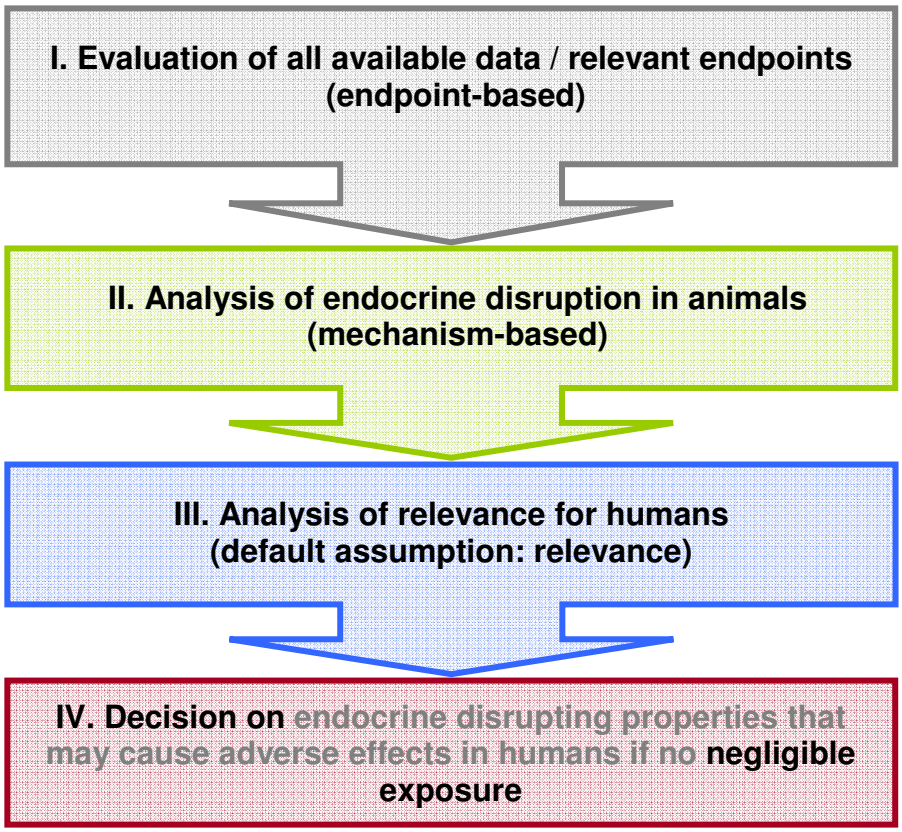

Figure 1: Modified tiered approach. For the original suggestion see Annex V. This tiered approach represents an overview and reflects the way a regulatory decision process may be made in general. A more detailed decision tree is shown in Figure 2.

Step IV

The fourth and last step consists of the decision whether a substance would have to be regarded as an endocrine disruptor in a regulatory sense. If, at this stage a substance is considered to have endocrine disrupting properties that may cause adverse effects in humans, the final decision on approval or disapproval should be made, based on assessment of exposure. Approval 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. In this context, the need for a more science-based definition of negligible exposure, taking into account concepts like the margin of exposure (MOE) should be pointed out.

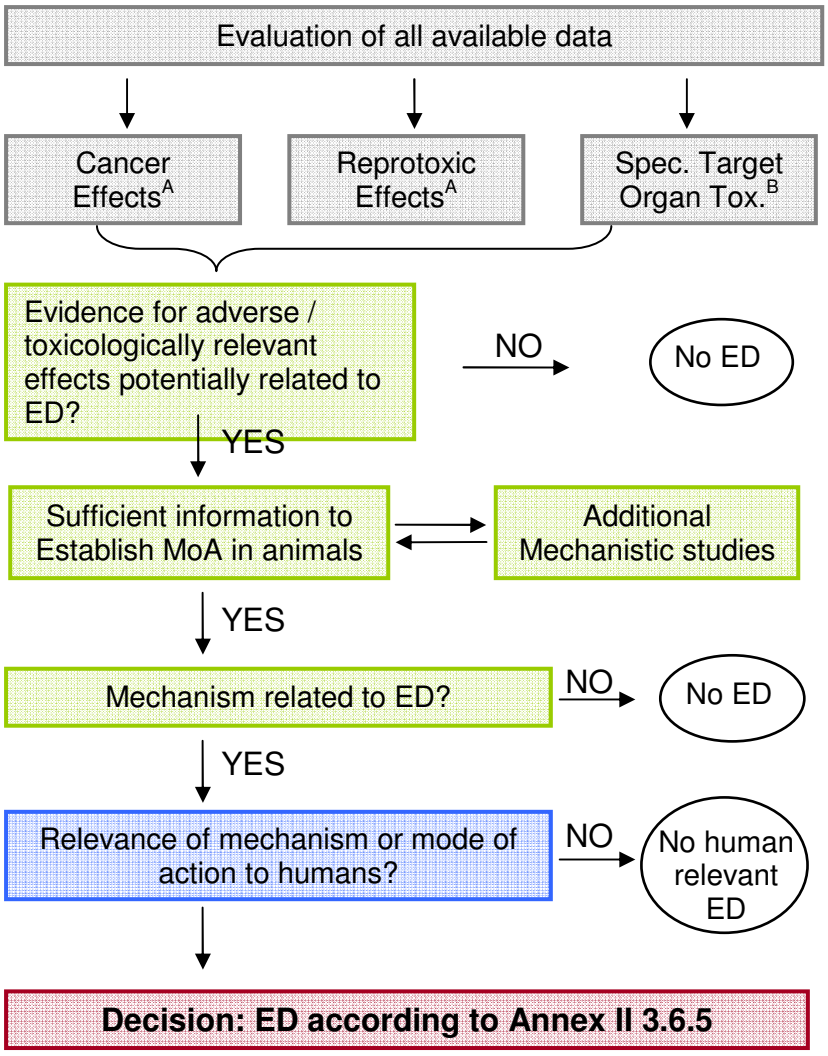

Figure 2: Modified detailed draft decision tree. This decision tree is a more detailed elaboration of the tiered approach suggested in Figure 1. Colours of boxes represent the relation to the four steps of the tiered approach shown in Figure 1. After evaluation of all data an endpoint based hazard analysis 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 regulatory sense in the last step (red boxes). For a detailed description of the decision process see text. 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) can occur after single exposure (SE) or repeated exposure (RE) according to Reg. (EC) No. 1272/2008. 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 its target organs, including the immune- or the nervous-system, may be regarded as being of particular importance.

Discussion

The discussion on criteria and the decision tree in the plenary sessions and breakout groups resulted in the following recommendations (for more details see results of the breakout groups in Annex IV. The original decision tree is presented in Annex V):

- The decision tree was considered useful (with modifications).

- It was recommended that toxicological relevance should be part of the decision on adversity (as modified in Figure 2).
- It was suggested to change the position of step II (original mechanism-based analysis of endocrine mechanism in animals) and III (original endpoint based analysis of all relevant endpoints) so that the endpoint-based analysis is translated into a mechanism-based analysis.
- It was suggested not to exclude substances labelled CMR categories 1A or 1B from the decision process for allowing mechanism based analysis.
- The use of mechanism of action was preferred to mode of action (mode of action does not have to be replaced).
- The default assumption on human relevance is that findings in animal studies are relevant to humans. For a detailed discussion on human relevance see results of breakout group IV.
- It was advised to regard each effect/endpoint separately within the decision process. Although a single endpoint, e. g. thyroid tumour development under inducers of thyroid hormone metabolism in rodents, may be considered to be less relevant to the human situation, other effects/endpoints exerted by the same substance during a critical window of development may indeed also be critical to humans.
- It was recommended to add a question to the boxes on mechanism that specifically asks: 'Is there a relationship to an endocrine mechanism?'

  If no, the substance is not regarded as an ED and an exit should be provided at this point. If yes, proceed to the next step. If uncertain, ask for more data or default to ED.
- It was recommended that prior to its adoption, the decision tree should be tested with a higher number of compounds including known positive ED compounds and known negative controls.

4. Summary and recommendations

The workshop discussions have provided recommendations for criteria for regulatory assessment of active substances in plant protection products with potential for adverse effects on the human endocrine system. Furthermore, suggestions were made for the improvement of the conceptual framework for evaluating potentially adverse endocrine effects and their relevance for humans under realistically proposed exposure conditions, which was presented by the BfR. The decision tree was considered useful for the scientific assessment of active substances in plant protection products with potential to cause adverse effects on the human endocrine system to be adopted in accordance with the regulatory procedure. It was strongly recommended by the majority of workshop participants to replace the preliminary interim criteria implemented in the regulation (EC) No 1107/2009 in the decision making process as soon as possible by specific scientific criteria. Among the discussed criteria, adversity was the least controversial. WHO/IPCS definitions for adversity and endocrine disruptors, respectively, were favoured by a majority of participants as working definitions for the meeting.

Besides assessment and decision criteria, the importance of extending dosing in toxicity studies to the low dose range and to acknowledge critical windows of development as well as a potential need to refine current testing guidelines and study designs were critically discussed, but no consensus was reached. Both the necessity for research to test the robustness and scope of effects in the low dose range as well as the need to integrate further endpoints, including developmental neurotoxicity or developmental immunotoxicity, in toxicity testing of substances with endocrine disrupting properties, were emphasised.

Since a definition of negligible exposure is crucial for decision making, different definitions of negligible exposure were considered. At the workshop, a more science- based definition was favoured over the default value of 0.01 mg/kg food laid down in the new plant protection products regulation.

While these recommendations might be implemented within a conceptual framework for a tiered decision process on substances with endocrine disrupting properties as a starting point for the development of measures to be adopted in accordance with the regulatory procedure (see Figures 1 and 2), there were also other suggestions which may be integrated into future international projects (e.g. validation of methods for the detection of low dose effects) and may be considered in future decision processes. The recommendations for next steps are summarised below.

1. Presentation of the conclusions of the workshop to the European Commission and EFSA
2. Publication of the workshop scientific results and the conclusions of the workshop by the organising committee
3. Discussion of the recommendations and proposed next steps of the workshop together with public, risk managers, NGOs and other stakeholders, e. g. at a BfR forum in 2010
4. Consideration of the relevance of this conceptual framework for a tiered approach by OECD and other bodies, e. g. IPCS/WHO
5. Search for and compilation of practical examples of endocrine disruption that were not detected in routine studies

Recommendations for research and further international projects in the future were:

1. to consider the extension of existing guidelines to better address the issue of ED
2. to incorporate an appropriate number of dose levels in study designs to determine a dose-dependency for relevant endpoints if low dose effects proved to be robust
3. to concentrate on developmental exposure and life-time assessment
4. to include research on detection of „changes in state“ that render an organism more susceptible to environmental influences
5. to hold workshops addressing the low dose issue and encourage experimental work to clarify the relevance of or clearly establish reproducible effects in the low dose range

Annex I: Workshop programme

Risiken erkennen – Gesundheit schützen

Wednesday, November 11th 2009

14.00	Shuttle transport Steglitz International Hotel – BfR	
15.00 - 15.10	**Welcome**	Ursula Banasiak, BfR
15.10 – 16.00	**Introduction and workshop outline**	Karen Hirsch-Ernst, Philip Marx-Stoelting, BfR
16.00 - 16.20	**The Community strategy for endocrine disruptors – status and priorities**	Patrick Murphy, EU Commission
16.20 - 16.40	**Coffee break**	
16.40 - 17.20	**Informative value of methods**	Remi Bars, ECETOC
17.20 - 18.00	**Targets and mechanisms of ED**	Jun Kanno, NIHS
18.00 - 18.40	**Dose relevance**	Jerry Heindel, NIEHS
18.40 - 19.20	**Human relevance**	Ellen Silbergeld, JHSPH
19.20 - 19.35	**Using mode of action information to improve regulatory decision making**	Richard Lewis, Syngenta
19.45	**Get together**	
21.30	Shuttle transport BfR – Steglitz International Hotel	

Thursday, November 12th 2009

08.45	Shuttle transport Steglitz International Hotel – BfR	
09.30 - 10.00	**Plenary session,** **Break out group briefing**	Moderation: Roland Solecki, BfR
10.00 - 10.15	**Coffee break**	
10.15 - 12.30	**Break out groups: Answering the questions**	
12.30 - 13.30	**Lunch break**	
13.30 - 15.00	**Break out groups: Presentation and discussion of the case studies**	
15.00 - 15.15	**Coffee break**	
15.15 - 16.00	**Break out groups: Discussion of recommendations and next steps**	

Thursday, November 12th 2009, continued

16.00 - 16.30	**Coffee break**	
16.30 - 18.30	**Plenary session: Reports from the break out groups**	Moderation: Jochen Buschmann, ITEM and Istvan Sebestyen, EFSA
16.30 - 17.00	**Break out group I: Informative value of methods**	
17.00 - 17.30	**Break out group II: Targets and mechanisms of endocrine disruption**	
17.30 - 18.00	**Break out group III: Dose relevance and criteria for adverse effects in animal studies**	
18.00 - 18.30	**Break out group IV: Human relevance of evidence for ED**	
18.30	Shuttle transport BfR – Steglitz International Hotel	
19.30	Public bus service Hotel – Dinner	
20:00	**Dinner**	

Friday, November 13th 2009

08.45	Shuttle transport Steglitz International Hotel – BfR	
09.30 – 10.15	**Summary of the results**	Karen Hirsch-Ernst
10.15 – 10.30	**Coffee break**	
10.30 – 12.00	**Plenary discussion**	Moderation: Helen Hakansson, Karolinska Institute and Ibrahim Chahoud, Charité
12.00 - 12.30	**Concluding remarks and potential next steps**	Karen Hirsch-Ernst, Roland Solecki, BfR
12.30	**End of workshop (optional: lunch break)**	
13.15	Shuttle transport BfR – Steglitz International Hotel	

Annex II: List of participants

List of Participants

Risiken erkennen – Gesundheit schützen

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Annex III: Results of the questionnaire

In preparation for the workshop a questionnaire was sent out to invited experts. Aims of the questionnaire were to facilitate and structure discussion at the workshop and to identify issues on which experts confined high level of agreement and consequently, to select controversial questions for discussion. The questionnaire was also aimed to integrate experts' opinion into the BfR draft concept for assessment and decision criteria on plant protection products with endocrine disrupting properties at an early stage. Of the questionnaires sent out 33 (~75%) were returned in time for analysis prior to the meeting (by October 23, 2009). Since some participants were nominated for the workshop later, a few questionnaires were sent out for informational purposes only and were not included in the calculations at the present state. From the results of the survey, an index of agreement (IA) was calculated for each question for which three options were given according to the following formula:

$$IA = (Yes - No) / (Yes + No + Undecided) \times 100 [\%]$$

It is suggested to focus on questions with an IA of below 50% and to regard questions with an IA of above 50% as less controversial. The results of the questionnaire, the IA, selected comments from participants on single questions and suggestions for further action are presented in the summary below.

Question 1: Assignment to breakout groups

Assignment of breakout groups will be presented in a different document.

Question 2: Which definition of ED do you prefer?

A majority of 24 participants chose Weybridge or IPCS as the preferred definition of ED. Since these definitions differ only slightly (i.e. IPCS mentions subpopulations), the IPCS definition was chosen as a working definition. The IA was not calculated, since more than three options were given here.

Comments from single participants:

- Any definition is preferred, as long as it also defines "adverse".

Action suggested: IPCS definition is proposed to be regarded as working-definition. No detailed discussion foreseen.

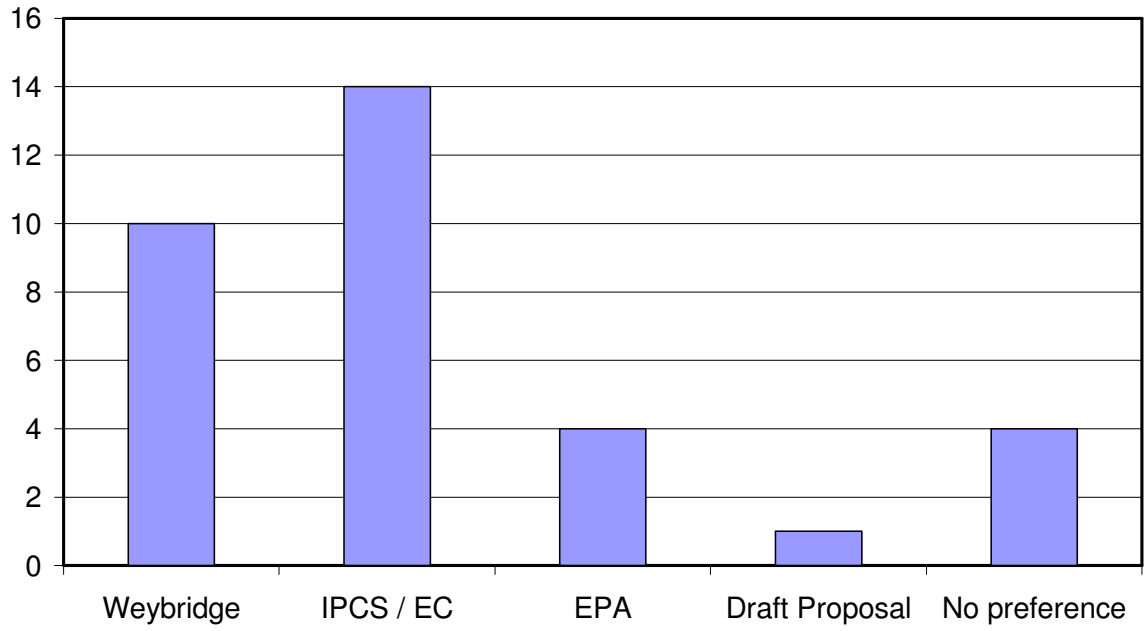

Figure 1: Results for the question on preferred definition for ED. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Question 3: How would you define a low dose effect?

Experts had diverging opinions on definition of low dose as presented in Figure 2. The IA in favour of realistic human exposure level as the definition is 20%.

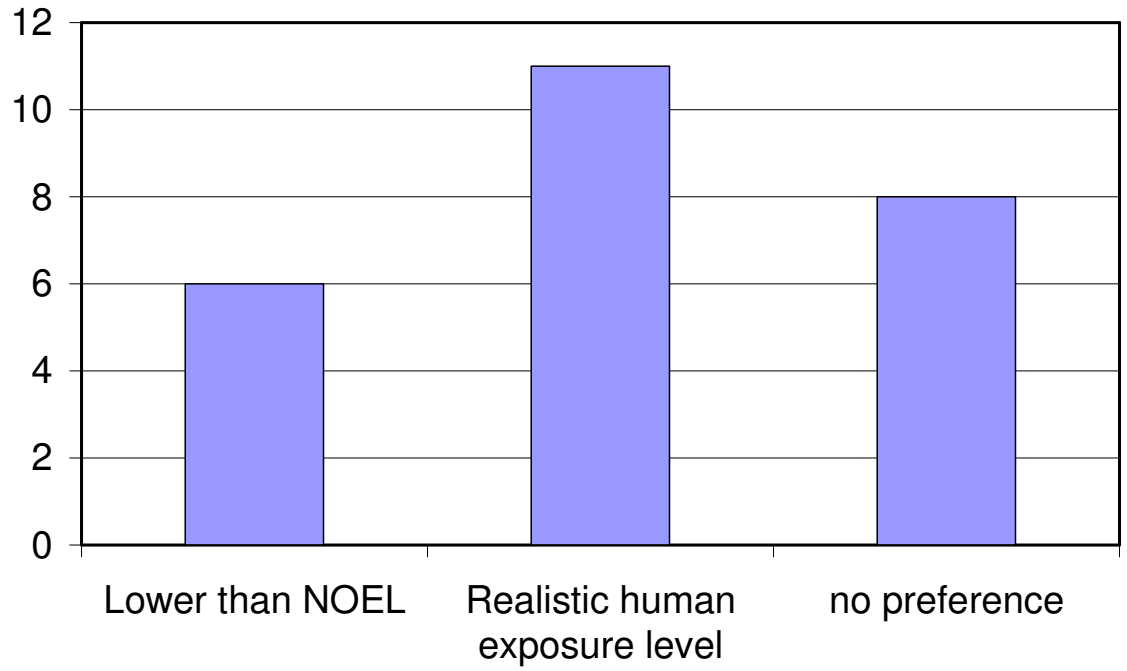

Figure 2: Results for the question on preferred definition for low dose. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments/suggestions from participants:

- A low dose effect is an effect occurring in the µg or even the ng range.
- An effect can only be lower than the NOEL on a limited dose-response curve.
- It is wise to choose a working definition, in part because it is sometimes difficult to know or estimate with precision the level to which humans are exposed.

Action suggested: Should be discussed in breakout group III. In addition a regulatory and / or political decision on how to integrate low dose in current international guidelines and protocols for toxicity testing is required.

Question 4: What is a negligible exposure level?

Several options were given. Three major ones can be summarized: negligible is below a defined value (e.g. 0.01 mg/kg foodstuffs as given in Directive EC 396/2005) or the TTC-(threshold of toxicological concern)-concept is applied or the MOE (margin of exposure). Participants were undecided about the best concept to be applied as presented in Figure 3 below. However, MOE and TTC were favoured over the defined maximum residue level given in Directive EC 396/2005 and as provided in the new European plant protection products legislation. Since more than three options were given, no IA was calculated here.

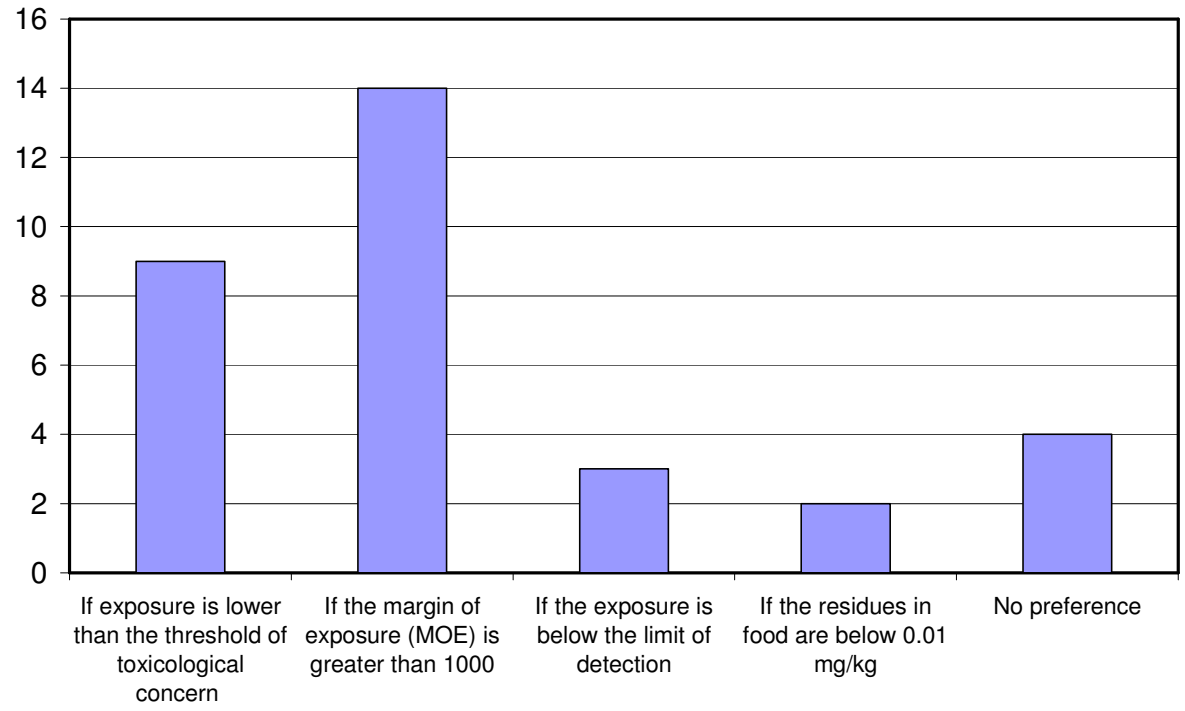

Figure 3: Results for the question on negligible exposure. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants:

- If a chemical is not tested, then the threshold for concern is not known.
- Negligible exposure level could mean different things such as exposure to a very limited population but also that the chemical is handled so the emission is kept to a minimum.

Action suggested: Since negligible exposure is a critical term in the new legislation, there is a need for discussion. Therefore, this question should be discussed in breakout group IV.

Question 5: How can 'adverse' be defined?

Definition of adversity is crucial if adversity is to be considered as one important criterion for decision on plant protection products with endocrine disrupting properties. A majority of responders favoured ECETOC and EPA definitions.

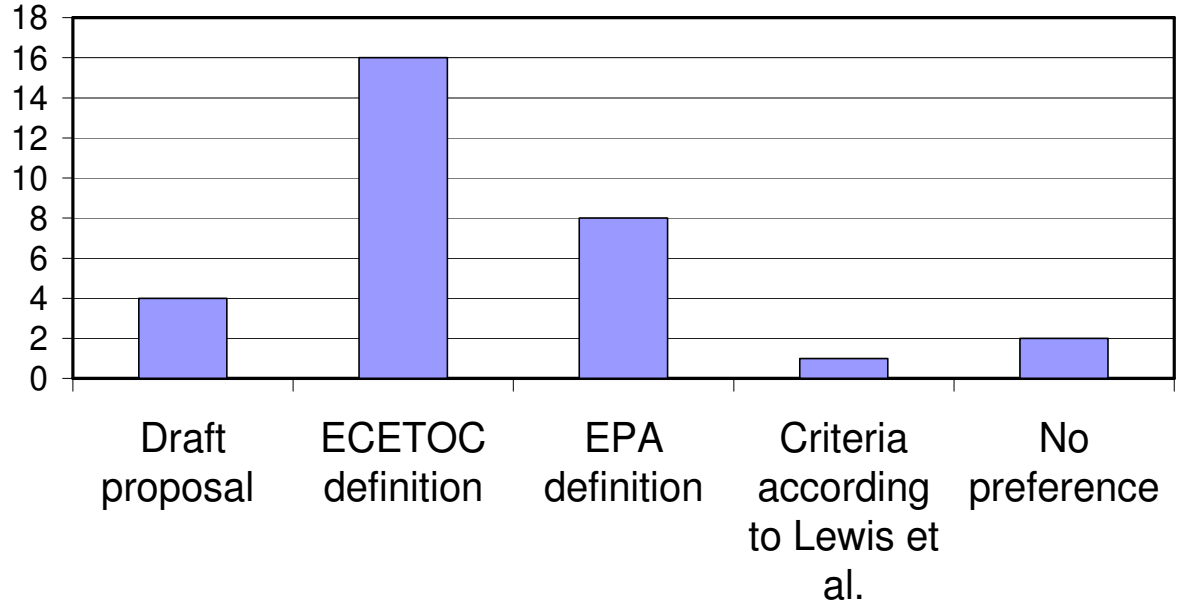

Figure 4: Results for the question on preferred definition for adverse. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants:

- OECD/IPCS definition is preferred (this comment was given by several participants).
- Consideration of the definition in the US NRC Toxicity Testing in the 21st Century is recommended.

(IPCS definition: A change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase insusceptibilitiy to the harmful effects of other environmental influences.)

Taken the results and comments together, the following synthesis of definitions is suggested: *A biochemical change, functional impairment, or pathological lesion (in response to a stimulus) that either singly or in combination affects the performance of the whole organism or reduces the organism's ability to respond to additional environmental challenge.*

Action suggested: To be discussed and decided during the workshop. Should be discussed in detail in breakout group III. A synthesis of ECETOC / EPA and IPCS may be suggested.

Question 6: Are the core studies laid down in point 5.3, 5.5 and 5.6 of Table 1 sufficient to provide any evidence on potential effects on the endocrine system in mammals?

A majority of experts agreed, that the core studies on short-term toxicity, reproductive and developmental toxicity, long term toxicity and carcinogenicity are sufficient to provide any evidence on effects on the endocrine system in mammals. However, the IA equalled 48.4%.

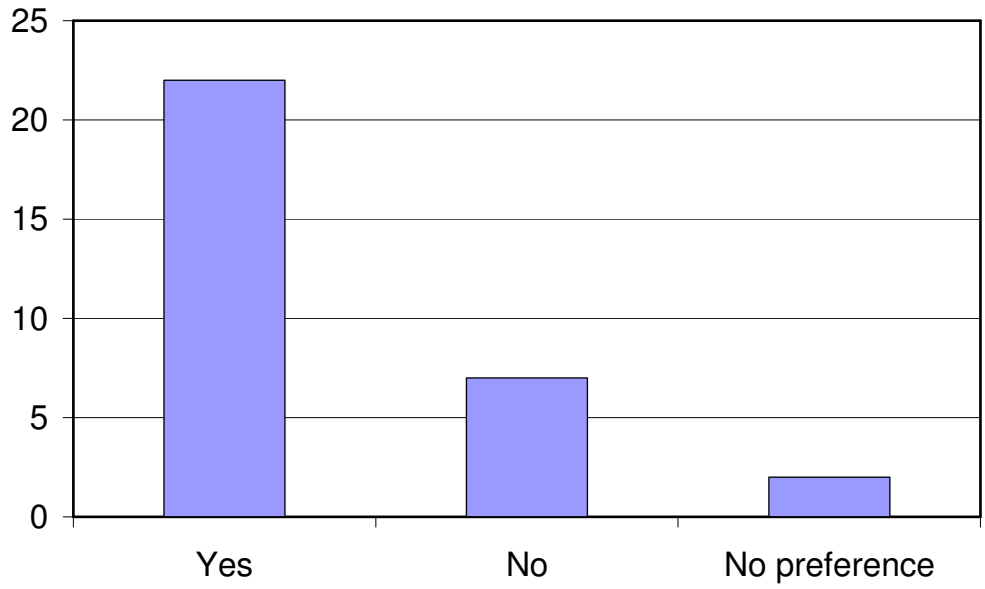

Figure 5: Results for the question on core studies. N=33; deviations from this number can be explained by some participants who did not answer this question at all. IA =48.4%.

Comments from participants:

- If you mean that the core studies will provide evidence on potential effects on the endocrine system for any type of endocrine toxicity my answer is No.
- More end-points, including high throughput data/signatures are needed in addition to the existing studies.
- The two generation reproductive effect studies of modern design that incorporate endocrine sensitive endpoints are the most useful of required studies.

Action suggested: Should be discussed in breakout group I.

Question 7: Is a stepwise approach feasible that, triggered by evidence observed in core studies mentioned, additional mechanistic studies will be required to clarify the potential endocrine mechanism and its relevance to humans?

Participants basically agreed that a tiered approach is feasible. The index of agreement was 75%. The development of a more detailed draft decision tree and its discussion will therefore be a major scope of the workshop.

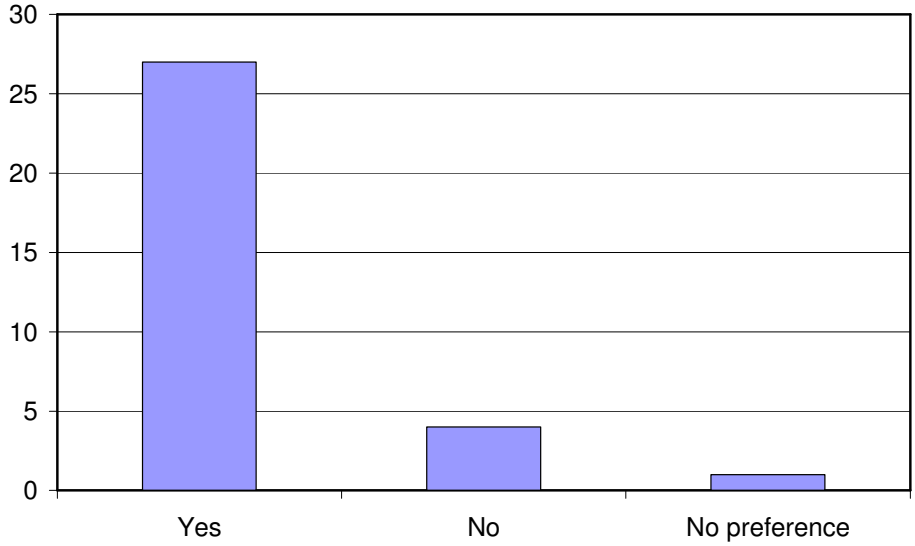

Figure 6: Results for the question on a stepwise/tiered approach. N=33; deviations from this number can be explained by some participants who did not answer this question at all. IA=75%.

Comments from participants:

- Current tests are not fully adequate and they should be augmented with more sensitive screens.

Action suggested: A stepwise approach is favoured by a vast majority of participants. BfR will therefore be suggesting a more detailed draft decision tree to be tested on some examples and to be discussed during the workshop.

Question 8: Are existing study designs comprehensive enough to detect endocrine effects?

16 of 33 responders answered 'yes' (see Figure 7 below).

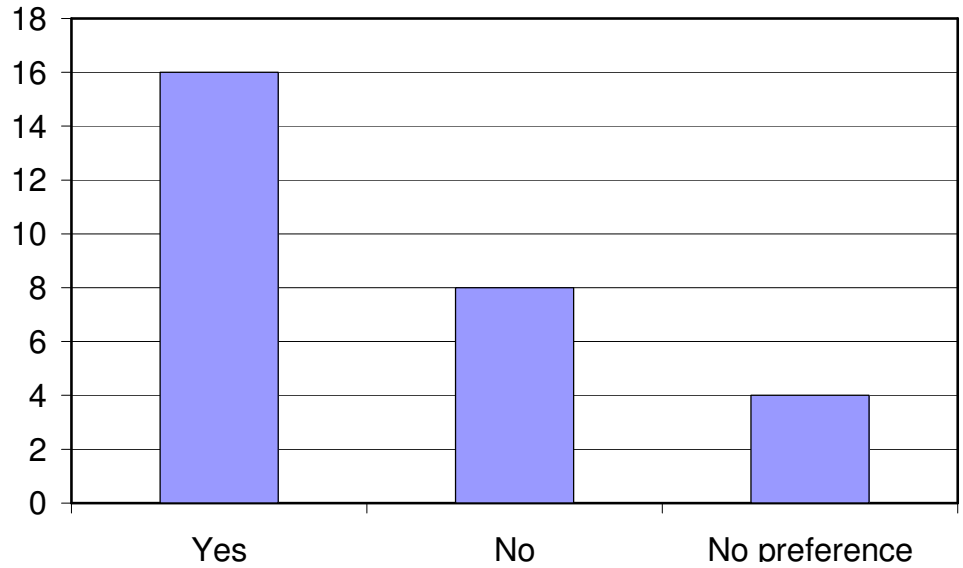

Figure 7: Results for the question on existing study designs. N=33; deviations from this number can be explained by some participants who did not answer this question at all. IA=42.3%.

Comments from participants:

- There are existing study designs, but not necessarily the test guidelines of OECD or similar organisations.
- More knowledge is needed to understand the links between early developmental landmarks and functional consequences.
- Rephrase: comprehensive enough to detect effects that may result from endocrine mechanisms.
- Existing tests still do not do so adequately. Some, like the two-generation mammalian test, can be improved by adding endocrine sensitive developmental endpoints such as time to vaginal opening and preputial separation or anogenital distance.

Action suggested: Should be discussed in breakout group I.

Question 9: Is it appropriate to acknowledge cumulative effects, resulting from exposure to substances displaying a common mode of action or targeting the same hormonal system in the regulatory framework?

Approximately three quarters of responders agreed that acknowledging cumulative effects is important. The IA was 57.1%.

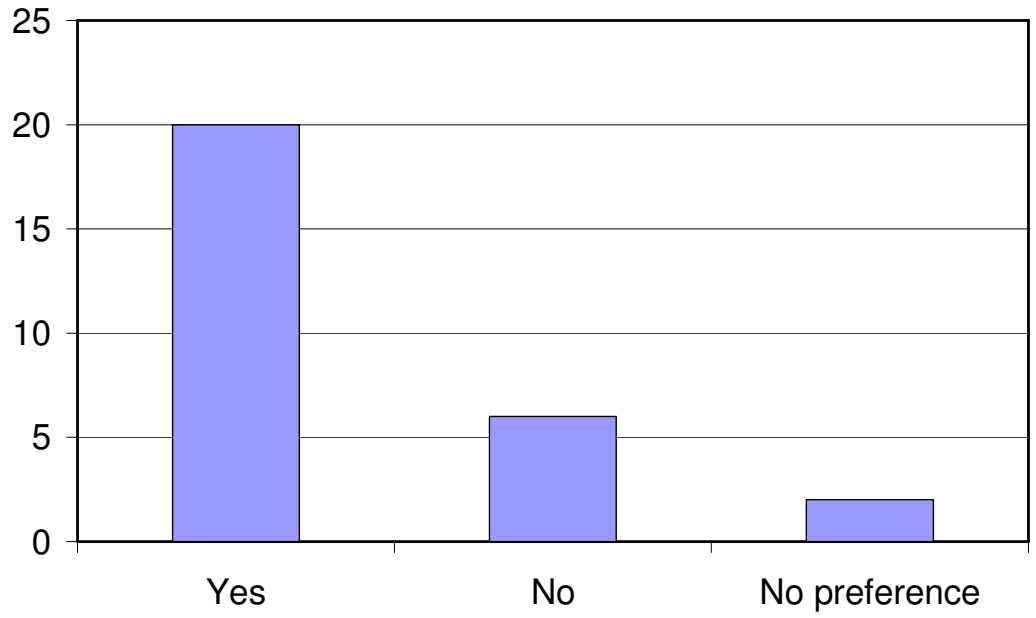

Figure 8: Results for the question on acknowledgement of cumulative effects. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants:

- It has to be a case-by-case decision.
- Cumulative risk assessment is not solely based on hazards, but takes both hazard and exposure into account. If substances are disapproved purely due to their hazard potential, cumulative risk assessment for endocrine disrupting pesticides in food will only need to be performed for mixtures with individual residues below 0.010 mg/kg foodstuff.
- If we are going to be adequately protective of human health we must reflect these in our risk assessments to the extent possible.

Action suggested: Since criteria for single substances have to be developed prior to discussing how to acknowledge cumulative effects of several substances, it is suggested to leave this topic open for future discussion at a different workshop.

Question 10: Can adversity be used as a criterion to analyse potential endocrine disrupting properties?

Adversity was the criterion for assessment of and decision on plant protection products with potential endocrine disrupting properties for which the highest level of agreement was obtained. The IA was 73.3%.

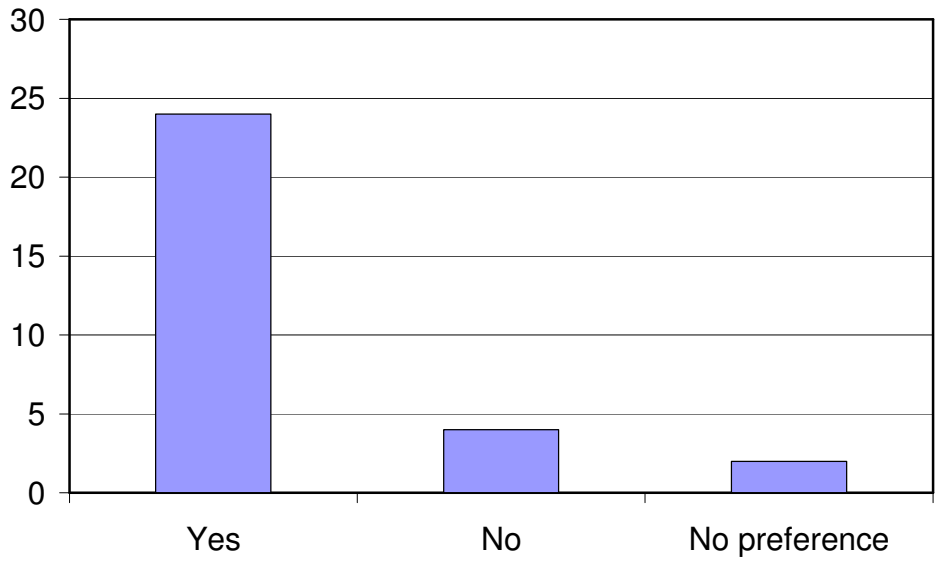

Figure 9: Results for the question on adversity as a criterion for assessment and decision on ED. N=33; deviations from this number can be explained by some participants who did not answer this question at all. IA=73.3%.

Comments from participants:

- No, recognition of 'adversity' may change rapidly in accordance with increment in knowledge of basic biology.
- Anything that is not adverse is also not disrupting.

Action suggested: Adversity should be integrated into the detailed draft decision tree to be suggested during the workshop.

Question 11: Can specificity (direct effect versus secondary, indirect effect) be used as a criterion to analyse potential endocrine disrupting properties?

23 out of 33 answered 'yes' (see Figure 10 below). Consequently specificity will be integrated into a preliminary draft decision tree. However, since the IA was only 43.8% and a definition of specificity will have to be decided on, further discussion during the workshop is regarded as necessary.

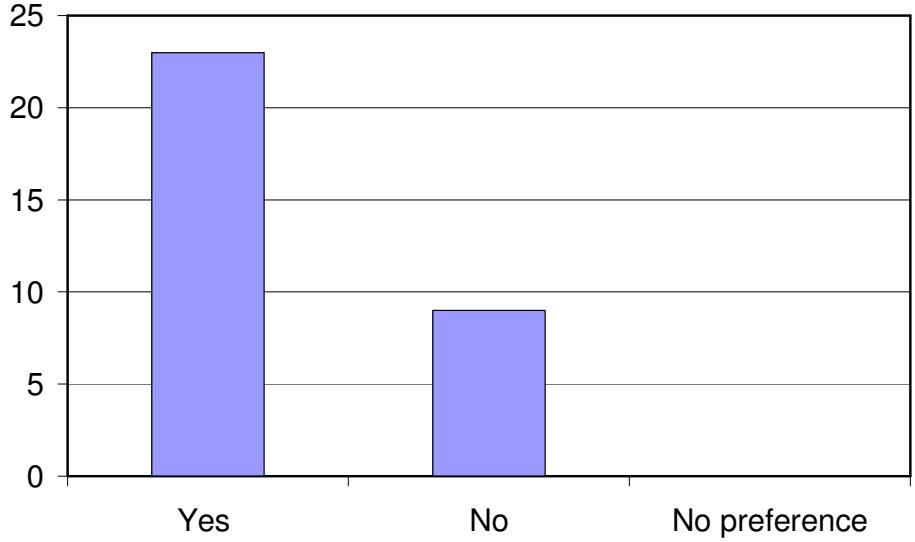

Figure 10: Results for the question on specificity as a criterion for assessment and decision on ED. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants:

- Endocrine disruption may itself be secondary.
- Does it matter to the individual if the damage stuns from a secondary/indirect effect?

Action suggested: Should be discussed in breakout group II. Potentially to be integrated into a detailed draft decision tree.

Question 12: Can dose dependency be used as a criterion to analyse potential endocrine disrupting properties?

20 out of 33 responders answered 'yes' (as presented in Figure 11 below). Since the IA was 34.4% only, further discussion at the workshop is regarded necessary.

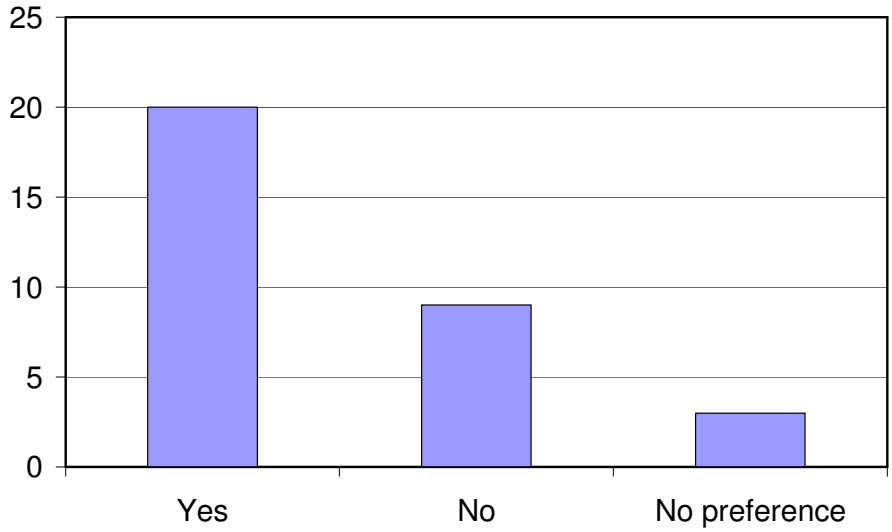

Figure 11: Results for the question on dose dependency as a criterion for assessment and decision on ED. N=33; deviations from this number can be explained by some participants who did not answer this question at all. IA=34.4%.

Comments from participants:

- Chronic adverse effects generated by low dose endocrine effect can be a result of multi-step epigenetical alterations, so that the response can be similar to the results seen in carcinogenesis studies, that is, not all animals respond exactly equally but respond in a probabilistic way, such as increase in incidence.

- Yes, for a single action. No, for a combination of actions (u-shaped curve?).

Action suggested: Should be discussed in breakout group III.

Question 13: Is it justified from a scientific point of view to use the preliminary criteria for ED mentioned in the draft regulation (category 3 carcinogen / category 3 reprotoxicant)?

Here a majority of participants answered 'no' (IA = -86,2%). It is therefore regarded as consensus among experts that from a scientific point of view the preliminary criteria for decision on the endocrine disrupting potential of substances to be used as plant protection products presented in the draft regulation are not appropriate. Consequently, the workshop is aimed to consider and suggest other criteria to be used in a tiered approach for assessment and decision.

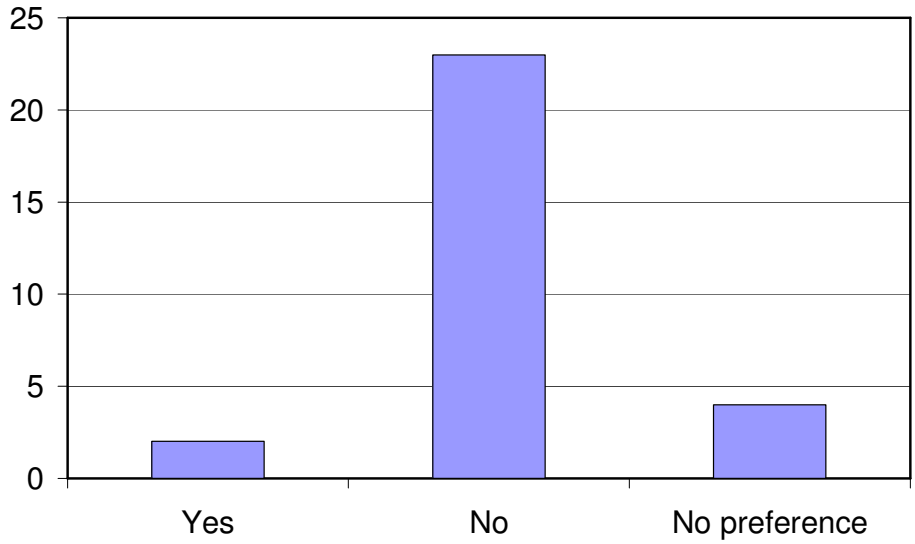

Figure 12: Results for the question on specificity the preliminary decision-criteria for ED presented in the draft regulation. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants:

- There are more than just 2 sets of endpoints regulated by endocrine mechanisms. Alternatively, is a cat3 carc and reprotox proof of an endocrine effect?

Action suggested: No further discussion foreseen since a good level of agreement was reached.

Question 14: Should a limit dose concept be considered?

A majority of participants answered 'yes' as presented in Figure 13 below. The IA equalled 28.6%.

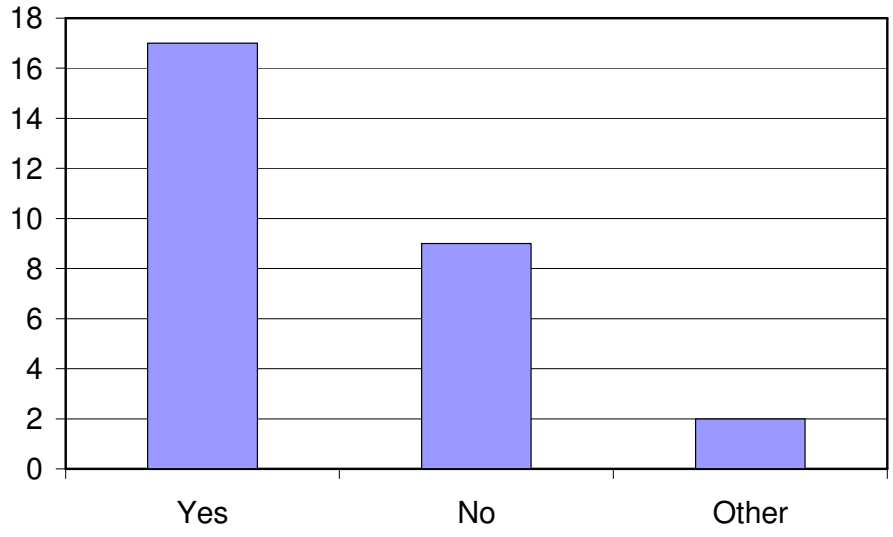

Figure 13: Results for the question on consideration of a limit dose concept. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants:

- In the absence of resolving the issues of low dose effects, this may not be appropriate.

Action suggested: A limit dose concept was considered acceptable by a slight majority. This topic should be discussed in breakout group II. Because regulatory and / or political decision will be required for integration of this concept into the European plant protection products legislation, it might be necessary to also address and discuss this issue in the future.

Question 15: Should we differentiate an alteration of endocrine function observed at dose levels causing general toxicity from that caused by a specific action on endocrine targets?

25 out of 33 responders answered 'yes' (see Figure 14 below). The IA equals 70%.

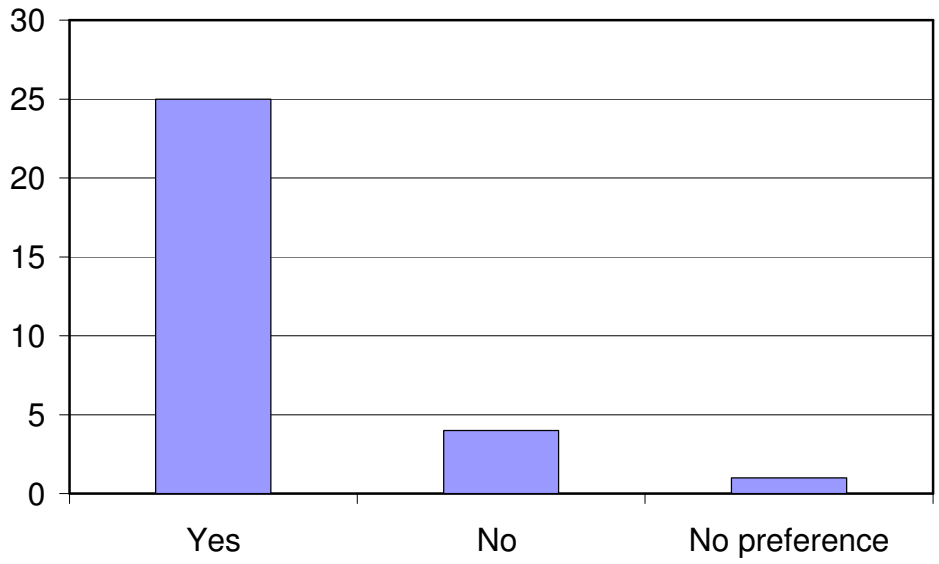

Figure 14: Results for the question on differentiation between alterations of endocrine function observed at dose levels causing general toxicity from that caused by a specific action on endocrine targets. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants:

- Yes, if we want to understand them. However, just because something happens at a generally toxic dose level does not mean it is unspecific.

Action suggested: No further discussion foreseen. The answer of experts might be seen as one recommendation of the workshop.

Question 16: Should adverse endocrine effects occurring at or above doses causing other general toxicity be regarded as relevant for the decision process?

A majority of participants answered 'no' as presented in Figure 15 below. Since the IA is -20.7%, there should be a discussion during the workshop.

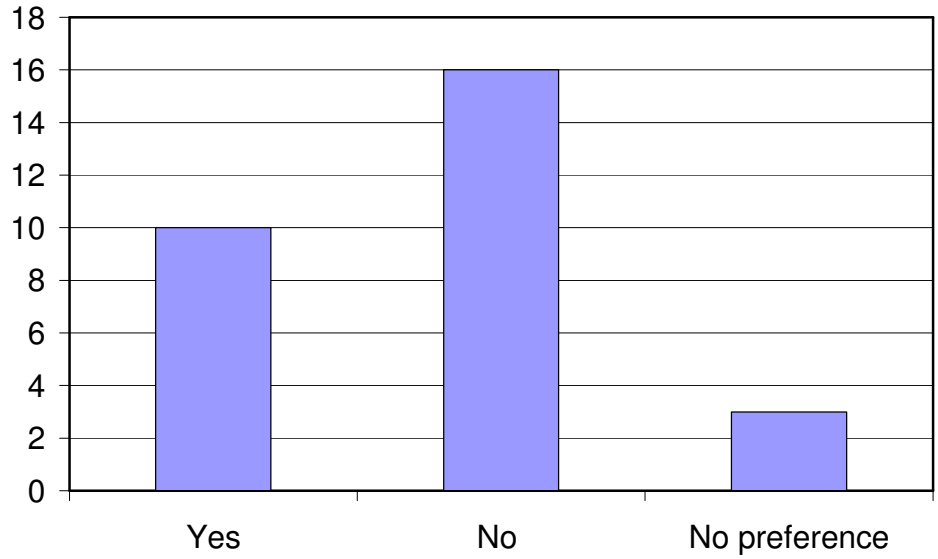

Figure 15: Results for the question on relevance of effects observed at dose levels of general toxicity. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Action suggested: Should be discussed in breakout group I or II.

Question 17: Can it be justified to limit the regulatory relevance of endocrine effects to the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-thyroid (HPT) axis?

Clearly, limitation of ED effects to only some parts of the endocrine system is not supported by workshop participants. Results to this question are presented in Figure 16. The IA is -59.4%.

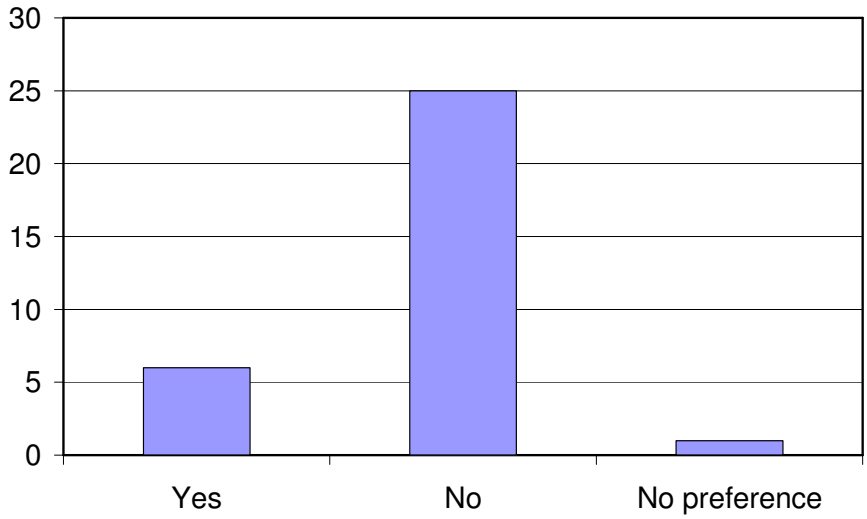

Figure 16: Results for the question on limiting ED to HPG and HPT axis. N=33; deviations from this number can be explained by some participants who did not answer this question at all. IA=-59.4%.

Action suggested: No further discussion foreseen. The clear answer of experts will be seen as one recommendation of the workshop.

Question 18: Will it also be necessary to address effects of other parts of the endocrine system such as the hypothalamic-pituitary-adrenal axis or the regulation of energy metabolism?

Since this question was closely related to the previous one, non-surprisingly here a majority of experts plead for acknowledging effects on all parts of the endocrine system, especially the Hypothalamic-Pituitary-Adrenal (HPA) axis and the regulation of energy metabolism. Results of this question are presented in Figure 17 below. The IA was 78.6%.

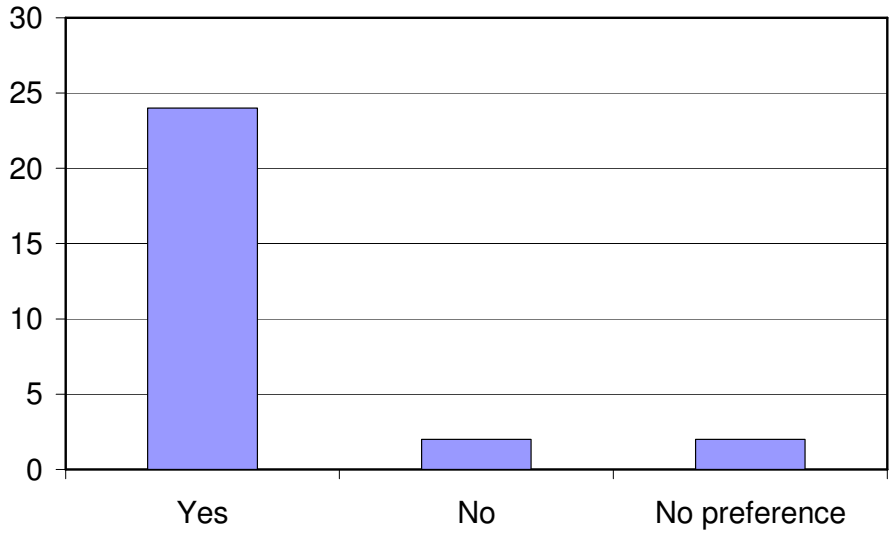

Figure 17: Results for the question if effects of other parts of the endocrine system will have to be acknowledged. N=33; deviations from this number can be explained by some participants who did not answer this question at all. IA=78.6%.

Comments from participants:

- Central nervous system is sensitive to, not only estrogenic/ antiandrogenic, but also to other analogs/ inhibitors of neurotransmitters.

Action suggested: The answer of experts might be seen as one recommendation of the workshop. However, since the extent of effects and mechanisms acknowledged will have to be discussed, this question is considered for breakout group II.

Question 19: Are endocrine effects relevant for acute toxicity and for setting an Acute Reference Dose?

Participants agreed as presented in Figure 18. The IA was 40.6%.

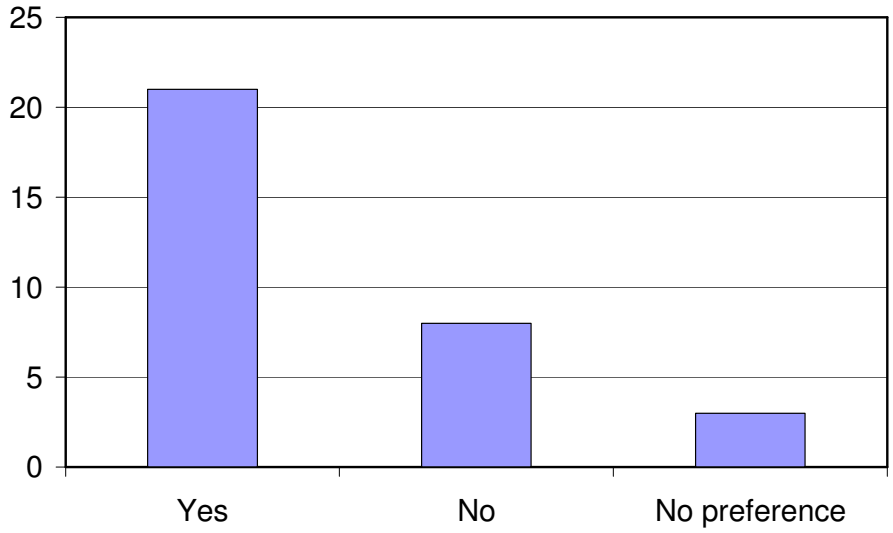

Figure 18: Results for the question on acute reference dose. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants: None.

Action suggested: Should be left open for future discussion, e.g. at another workshop focussing on ARfD.

Question 20: Should we classify and label substances with endocrine disrupting properties on the basis of existing categories (e. g for carcinogenicity, reprotoxicity or specific target organ toxicity), or is it necessary to adopt a new system for classification and labelling of endocrine disrupting substances?

A majority of participants agreed, that classification and labelling should be performed on the basis of the existing system (15 participants to 7 who preferred a new system). See Figure 19 below for an overview on answers. The IA was 30.8%.

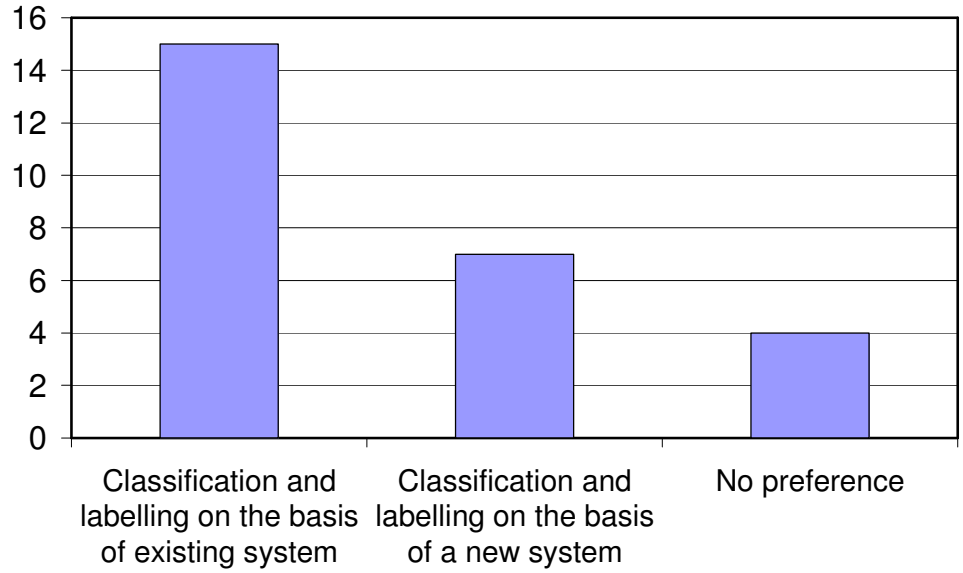

Figure 19: Results for the question on classification and labelling of endocrine effects. N=33; deviations from this number can be explained by some participants who did not answer this question at all. IA=30.8%.

Comments from participants:

- The existing classifications refer to endpoints rather than mechanism and can be used regardless of the mode of action which causes them.

Action suggested: Should be discussed in breakout group IV. Classification and labelling of substances is of great importance but since regulatory and / or political action is required this topic might also be left open for future discussion.

Question 21: Should newly discovered mechanisms of ED (e.g. hormone sensitisers) be integrated into the concept?

21 participants answered 'yes', while only a small number (4) answered 'no' or had no preference (4) as shown in Figure 20. The IA was high and equalled 60.7%.

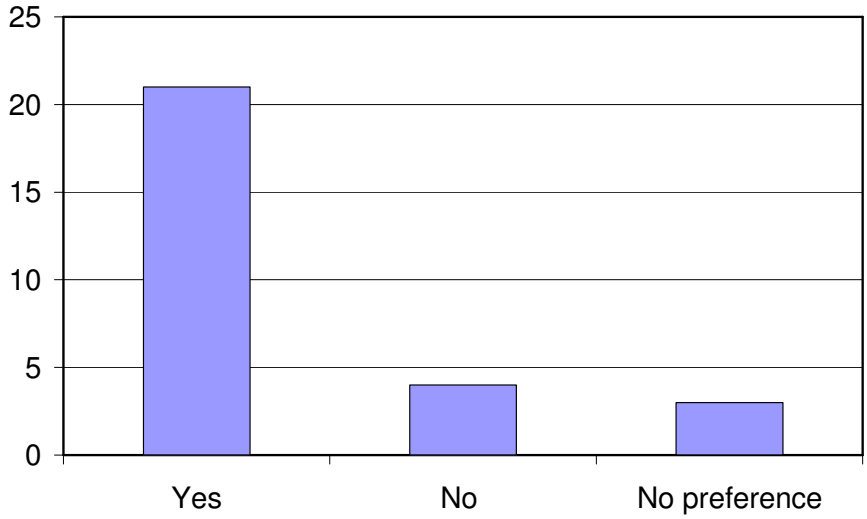

Figure 20: Results for the question on relevance of newly discovered (molecular) mechanisms of ED like hormonal sensitizers. N=33; deviations from this number can be explained by some participants who did not answer this question at all. IA=60.7%.

Comments from participants: None

Action suggested: Newly discovered mechanisms like hormonal sensitizers will clearly have to be acknowledged also for the sake of consumer protection. Since various mechanisms have been identified and more will surely be discovered as science progresses, this topic is suggested to be left open for future discussion, e.g. at another workshop on molecular mechanisms of ED.

Question 22: Is a mode of action analysis a feasible approach in the field of endocrine disruption?

A clear majority of participants agreed that a mode of action analysis is feasible also in the field of ED. 26 of 33 responders stated 'yes' while only 5 answered 'no' as presented in Figure 21 below. The IA was 67.7%, so mode of action is considered to be integrated into a draft detailed decision tree.

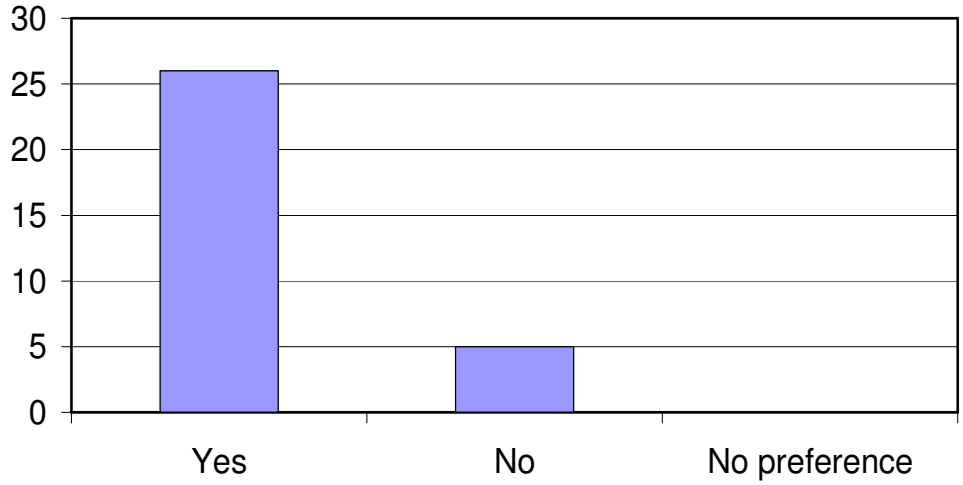

Figure 21: Results for the question on feasibility of mode of action analysis. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants:

- Yes, not only is it feasible, but it is important if we are going to perform risk assessments that incorporate cumulative aspects of co-exposure.

Action suggested: To be integrated into a more detailed draft decision tree.

Question 23: Is the IPCS mode of action framework (Boobis *et al.* 2008) feasible for analysing relevance to humans?

While a majority in general agreed on the IPCS MOA framework, nearly as many participants had no preference on this question. As a consequence the IA was low and equalled 39.3%.

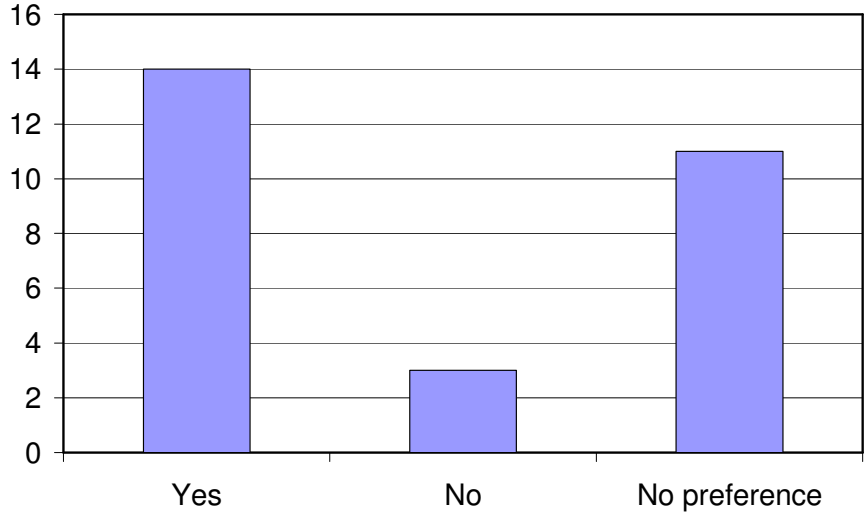

Figure 22: Results for the question on feasibility of the IPCS mode of action framework for ED effects. N=33; deviations from this number can be explained by some participants who did not answer this question at all.

Comments from participants:

- Yes, but should be updated with regard to endocrine effects.

Action suggested: Should be discussed in breakout group IV.

Annex IV: Results of the individual breakout groups

Breakout group I – Informative value of studies/methods

1st question for discussion: Are the core studies laid down in point 5.3, 5.5 and 5.6 of Table 1 sufficient to provide any evidence on potential adverse effects on the endocrine system in mammals?

- The groups answer was clearly YES, the core studies are sufficient to provide some evidence on potential adverse effects, but it is still not known, if all possible aspects are captured.
  - This is, however, not only a problem of ED, but also of other endpoints such as immunotoxicity etc.
  - It was speculated that interpretation of study-results might differ between researchers and regulatory agencies.
  - It was asked how to improve the methods and what could be new endpoints which could be included into existing guidelines.
  - Routine testing can not find all possible effects, therefore we have to ask scientist.

2nd question for discussion: Are existing study designs (OECD guidelines etc.) comprehensive enough to detect endocrine effects?

- There were concerns that the existing study designs are not comprehensive enough to detect adverse effects which might be caused by endocrine disruption in mammalian test organisms.
- However, the lack of concrete examples which are not captured by the test guidelines was criticized and research scientists were asked to come forward with such practical examples.
- Until practical examples have been provided, the question remains what additional endpoints should be included into existing guidelines?

3rd question for discussion: Should adverse endocrine effects occurring at or above doses causing other general toxicity be regarded as relevant for the decision process?

- Endocrine effects at clearly higher doses than those causing general toxicity might not be regarded as endocrine disruptive, if they are considered to be secondary. These substances should go to regular risk assessment under the EU PPP regulation.
- If we have evidence that endocrine disruption is the critical effect based on mechanistic studies, then YES.

- This question can't be solved, if the consideration of exposure in humans is excluded, e.g. by combining hazard identification and elements of risk assessment, in the decision making process.

Toxicological data for an example pesticide were provided and the criteria and decision tree were tested using this exemplary compound. The breakout group analyzed the example and found the criteria and decision tree useful. It was possible to establish a mode of action in accordance with the IPCS mode of action framework. Based on this mode of action it was decided that the effects observed would be relevant for humans. Based on the assessment and decision criteria it was also concluded that the example provided was an endocrine disruptor.

The following final recommendations were suggested by group I:

- Core principles upon which the group could agree:
  - The answers to the 3 questions
  - The example is an ED
  - A MoA could be established in the stepwise approach
  - The decision tree is considered useful
- Major issues of discussion and open questions were:
  - Do we expect „low dose" effects in reality for all ED?
  - What is negligible exposure for operators, bystanders, residents and consumers?
  - Different wording on ED under PPP and REACH
- Recommendations were:
  1. *Low dose workshop is recommended*
  2. *Go forward with the conclusion of the workshop to the commission.*
  3. *Consider extension of existing guidelines to better address this issue.*
- The next steps recommended were:
  1. More experimental work is needed to investigate and reproduce low dose effects.
  2. OECD and other bodies should also consider the relevance of this approach.
  3. Call for practical examples that were not detected in routine studies

Breakout group II – Targets and mechanisms of ED

1st question for discussion: Can specificity be used as a criterion to analyze potential endocrine disrupting properties?

- Criteria for specificity might be:

- The crucial issue of endocrine disruption was regarded generally as whether the effect is ultimately a receptor-mediated event.
- An effect should not be regarded as being non-specific or not relevant purely on the basis of being secondary. E. g., liver enzyme induction may lead to alterations in circulating hormone levels.
- In this context two new questions were raised: Can the dose at which an endocrine adverse effect is observed be used as a criterion for specificity? Can effects occurring at high doses (e. g at which general toxicity or other forms of toxicity occur) be regarded as relevant for the regulatory decision process?
- An endocrine adverse effect occurring above the dose level at which a different form of toxicity is observed, would be expected to be covered by regulation due to the fact that the apparently more sensitive effect (caused by other mechanisms of toxicity) would be the starting point for the derivation of reference values.

2nd question for discussion: Should a limit dose concept be considered?

- This question was clearly answered yes.
- The limit dose concept is already established in current testing protocols of animal apical studies (1000 mg/kg bw/d).

3rd question for discussion: Will it also be necessary to address effects of other parts of the endocrine system such as the hypothalamic-pituitary-adrenal axis or the regulation of energy metabolism?

- This question was clearly answered yes.

The following final recommendations were suggested by group II:

- There was concern that with current data sets, endocrine effects might be missed for certain compounds in the lower dose range, e. g. concerning particularly sensitive stages of pre-natal and neo- or postnatal development. These effects in the low dose range might turn out to be the most relevant from a health perspective. It was considered that, triggered by evidence, to encourage improvement of testing, particularly developmental toxicity testing in respect to the low dose range. (Amendment of existing guidelines).
- To avoid overlooking a particular effect that was mediated by a mechanism that operates at a low dose level (below a “traditional” NOAEL) potentially related to endocrine disruption, it will be necessary in regulatory toxicology to further develop,

validate and apply „new“ methodology (e. g. array approaches) to more comprehensively identify and interprete additional mechanistic (receptor mediated) effects.

Breakout group III – Dose relevance and criteria for adverse effects in animal studies

1st question for discussion: How to define adverse?

- Recommended definition (WHO/IPCS 2004): A change in morphology, physiology, growth, 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.
- Acceptable definition; some aspects are difficult to apply/implement with current TGs.

2nd question for discussion: Can dose dependency be used as a criterion to analyze potential endocrine disrupting properties?

- Yes, in principle; every biological effect is dose-dependent; under current TGs it is difficult to characterize non-monotonic dose response curves that are typical for hormonally active compounds. As a consequence if there is an indication of ED at any dose tested this should trigger examinations at lower dose levels/more appropriate dose levels and additional endpoints.

3rd question for discussion: What would be an appropriate low dose level in animal studies?

- Definitions on an absolute basis are not helpful; we need to have case-by-case decisions for each compound by taking into account kinetic information, human exposure levels, apparent NOAELs and mechanistic data

Toxicological data for an example pesticide were provided and the criteria and decision tree were tested upon this example. The breakout group analysed the example and found the criteria and decision tree useful with the following modifications:

- Biological relevance should be part of the decision on adversity; the box should be removed.
- We need to change the Box Mode of Action to Is there a relationship to an endocrine mechanism? If no, the substance is not an ED and an exit should be provided at this point. If yes, proceed to the next step. If uncertain, ask for more data.

The following final recommendations were suggested by group III:

- Major issues of discussion and open questions were:

- „Adaptive“ effects that go back to „normal“ and developmental delays may be a sign for ED; can short-term functional changes without long-term consequences be considered to be non-adverse?
- Biomarkers vs. adverse effects
- Need for confirmation of low dose effects? What happens at low doses vs. high doses of ED?
- Shapes of dose-response curves; low dose: to define or not?
- Future vs. existing data requirements; what data can we get already today?
- Evidence in vivo vs. in vitro

- Recommendations were:
  - Study designs need to incorporate appropriate number of dose levels to determine a dose-dependency for relevant endpoints.
  - For suspected ED use additional dose level(s) in main study (Generation study) to cover low dose range (TG requires a minimum of 3 doses)
  - Take dose-response curve into account, whether linear or non-monotonic
  - Concentrate on developmental exposure and life-time assessment
  - In the future, detection of „change in state“ needs to be included that render organism more susceptible to environmental influences
- The next steps recommended were:
  - Look into existing guidelines and guidelines presently under development to decide how practical improvements/new endpoints for all major endocrine tissues could be incorporated to detect ED
    - Sexual dimorphic development
    - Endpoints for neurohormonal toxicity
    - Endpoints for Metabolic Syndrome
    - Endpoints for HPA
  - In the data requirements if an extended one-generation study is conducted it should as mandatory include DIT and DNT cohorts.
  - Discuss this with the Commission

Breakout group IV – Human relevance of evidence for endocrine disruption

1st question for discussion: What is relevance to humans?

- **The default assumption is relevance;** it can be rebutted with additional data.
- Relevance is a qualitative call – at the first stage, not related to dose.
- Mechanism of action is the recommended context for determining relevance (draw upon example) - we recommend moving to a regulatory system that ensures availability of mechanistic data.[10]
- Experimental phenotype is not the basis for determining relevance by itself.
- A central problem is that the discussion is based on endpoints gained from the toxicity studies but that the definition of EDC is mechanism-based.
- For cancer and reproductive endpoints, decisions might be made as to relevance based on dose or phenotype, but for EDCs this is not feasible since endocrine systems are conserved across species.
- IPCS recommendations are not generally feasible since we do not usually have information on mechanism.
- Group IV proposed the following changes to the relevance for humans framework for the decision tree:
  - Step I: Look at the endpoints from standard tests for signals of ED including changes in distributions of the endpoint or increases in variability
  - Step II: Evaluation of relevant ED endpoints
  - Step III: Analysis of ED MoA (and mechanistic data if available)
  - Step IVa: if MoA for the endpoint is ED and relevant to endpoint in humans, then it is an EDC (and of high concern)
  - Step IVb: if the MoA is ED but endpoint affected in standard test not relevant to humans, it is still an EDC (and may be of high concern)
- Critical points:
  - Effect might be missed (e.g. low incidence) – size of studies
  - Look at effects on variability and ends of distribution
  - How do we consider adversity?
  - How do we consider potency?
  - Mechanism or mode of action? – mechanism should be basis for excluding relevance to humans

2nd question for discussion: Should we classify and label substances with endocrine disrupting properties on the basis of existing categories (e. g for carcinogenicity, reprotoxicity

[10] Mechanistic data are, however, already required by directive 91/414/EEC.

or specific target organ toxicity), or is it necessary to adopt a new system for classification and labelling of endocrine disrupting substances?

- In the next phase it was regarded to be necessary to develop a classification system for endocrine disrupting chemicals considering issues such as adversity etc.
- It was proposed to call it *Endocrine Disrupting Chemicals* or *Endocrine Toxicants.*
- It was asked whether a system possibly like carcinogens or reprotox – or a yes/no classification or level of concern should be recommended.
- A critical point was the question what to do about interim criteria.
    - Interim criteria are not scientifically based (to consider C3/R3 substances to be EDs)

3rd question for discussion: What is a negligible exposure level?

- Critical points: A MRL of 0.01 mg/kg food for all compounds is not a health-based scientific decision criterion.

Further topics discussed related to testing of substances with an endocrine disrupting potential:

- Obtaining information in the low dose range (important for EDCs)
- If the tests were not conducted within the known or estimated range of likely human exposure:
    - for existing compounds
    - 1st step: Biomonitoring to determine range of actual human internal dose was recommended
    - 2nd step: It was suggested to conduct limited toxicokinetics (or use kinetic data from respective studies available for plant protection products) in experimental species to establish exposure that produces this range of internal dose
    - 3rd step: Additionally it was proposed to conduct studies in this dose range.

    - new chemicals
    - It was suggested to use the apparent NOAEL from the standard tests to establish a dose range that is at least 1000 fold lower (learn from experience) for further testing.
    - This may be refined with further exposure data from producer or after chemical comes into use.
    - It was proposed to look for body wt and the effect observed or suspected based upon earlier data in the high dose range.

Toxicological data for an example pesticide were provided and the criteria and decision tree were tested upon this example. The breakout group discussed the example and found it of great value for discussion. The following recommendations were made with respect to the decision tree:

- Test the criteria and decision tree with known positive ED chemicals and known negative ED chemicals.

Annex V: Originally proposed decision tree

The following tiered approach and decision tree were suggested by BfR in preparation for the workshop. They were modified to account for recommendations made during the workshop. The originals are presented here to allow a better understanding of changes.

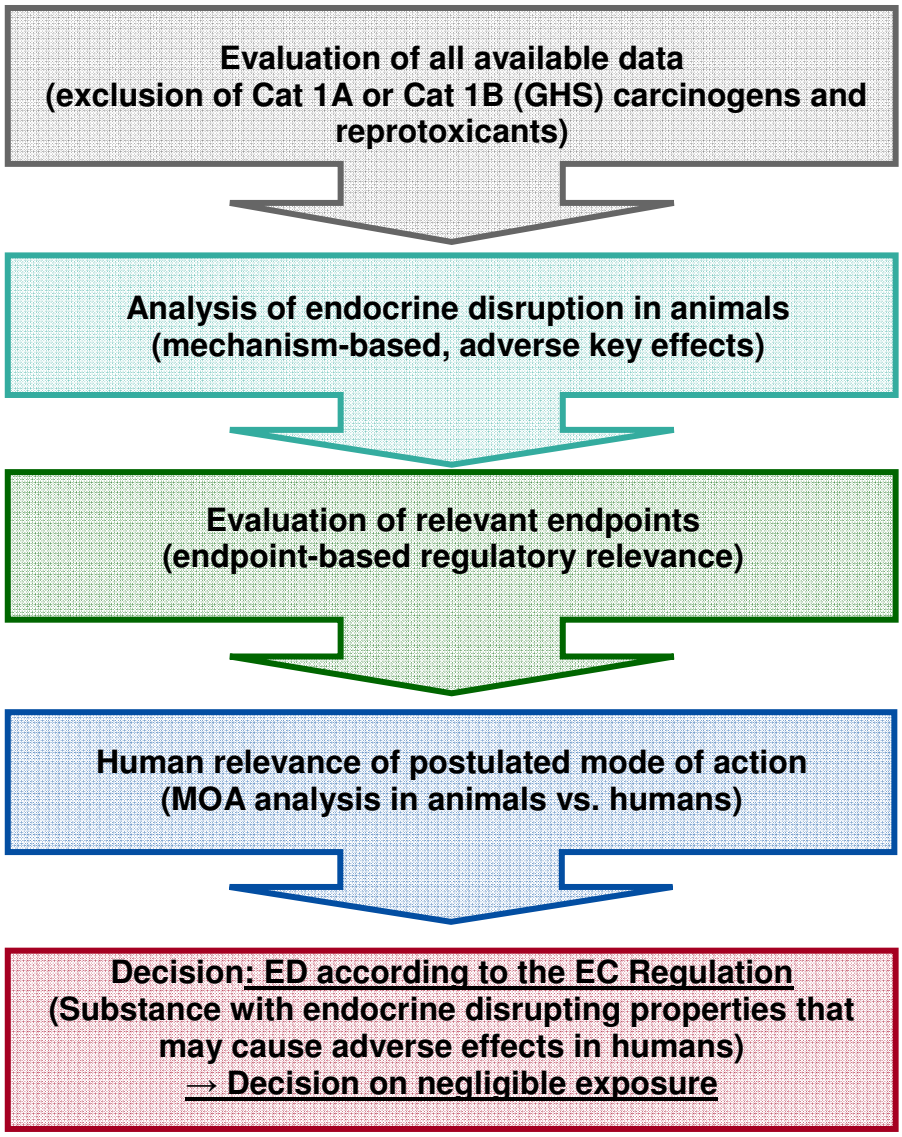

Figure 3: Originally proposed tiered approach. This approach consisted of five steps, starting with the evaluation of all available data and exclusion of cat 1A and 1B CMR substances according to the Globally Harmonized System (GHS) on Classification, Labelling and Packaging as laid down in Reg. (EC) No. 1272/2008. Prior to endpoint based analysis in step III a mechanism based analysis was suggested to be performed in step II. A mode of action analysis to determine human relevance was considered to be conducted in a fourth step before finally deciding on endocrine disrupting properties and negligible exposure in step five.

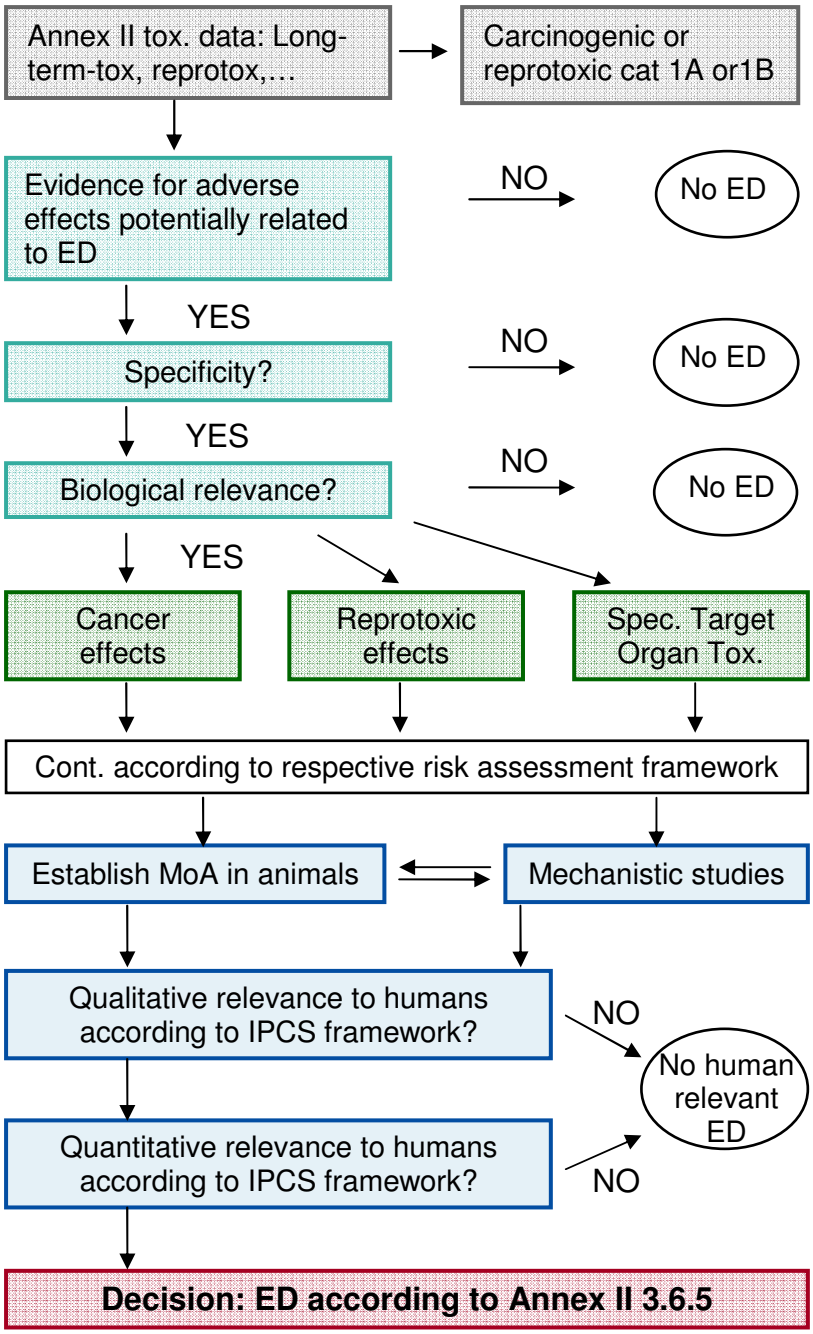

Figure 4: Original draft decision tree. This decision tree suggested several criteria for decision on ED including adversity, specificity, biological and human relevance. It also suggested integrating the respective IPCS framework into the decision on human relevance.

Annex VI: Abbreviations

ARfD	Acute Reference Dose
BfR	Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)
CMR	Carcinogenic, Mutagenic, or Reprotoxic
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
IA	Index of Agreement
IPCS	International Programme on Chemical Safety
MoA	Mechanism / Mode of Action
MOE	Margin of Exposure
MRL	Maximum Residue Levels
NGO	NON-Governmental Organisation
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NRC	National Research Council
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

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