



Endocrine Disruption

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Overview

1. 91/414 revision cut-off criterion
2. OECD ecotoxicology test methods
 - Conceptual framework
 - Screening assays
 - Definitive tests
3. Criteria for identifying endocrine disrupting properties
 - ECETOC guidance
4. Global considerations
5. Conclusions

91/414 revision cut-off criterion

- **Annex II, Article 3.6.5 (criteria for human health)**

An active substance ... shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, or other available data... it is not considered to have endocrine disrupting properties that may cause adverse effect in humans....

- **Annex II, Article 3.8.2 (criteria for the environment)**

An active substance ... shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines... it is not considered to have endocrine disrupting properties that may cause adverse effects on non target organisms unless the exposure ... under realistic proposed conditions of use is negligible.

Criteria

- Endocrine hazard has not been defined
- Four year period to define 'measures concerning specific scientific criteria for the determination of endocrine disrupting properties'
- Interim definitions
 - Considered to have endocrine disrupting properties
 - carcinogen category 3; and
 - toxic for reproduction category 3
 - May be considered to have such endocrine disrupting properties
 - toxic for reproduction category 3; and
 - toxic effects on the endocrine organs
 - No interim definition for ecotoxicology

Initiatives to define endocrine disruption

- ECETOC approach (toxicology and ecotoxicology)

- BfR approach (toxicology only)
 - Workshop to be held on November 11-13 in Berlin

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

Level 1

Sorting & prioritization based upon existing information

- Physical & chemical properties, eg MW, reactivity, volatility, biodegradability
- Human & environmental exposure, eg production volume, release, use patterns
- Hazard, eg available toxicological data

Level 2

In vitro assays providing mechanistic data

- ER, AR, TR binding affinity
- Transcriptional activation
- Aromatase & steroidogenesis in vitro
- Ah receptor recognition/binding
- QSARs
- High Through Put Prescreens
- Thyroid function
- Fish hepatocyte VTG screen
- Others (as appropriate)

Level 3

In vivo assays providing data about single endocrine mechanisms and effects

- Uterotrophic assay (estrogen related)
- Hershberger assay (androgen related)
- Non receptor mediated hormone function
- Other (eg thyroid)
- Fish vitellogenin assay (estrogen related)

Level 4

In vivo assays providing data about multiple endocrine mechanisms and effects

- Enhanced OECD 407 (endpoints based on endocrine mechanisms)
- Male and female pubertal assays
- Adult intact male assay
- Fish gonadal histopathology assay
- Frog metamorphosis assay

Level 5

In vivo assays providing data on effects from endocrine and other mechanisms

- 1 generation assay (TG415 enh)*
 - 2 generation assay (TG416 enh)*
 - Repro screening test (TG421 enh)*
 - Combined 28 day/repro screening test (TG422 enhanced)*
 - Partial and full life cycle assays in fish, birds, amphibians & invertebrates (developmental and reproduction)
- *Potential enh to be considered by VMG

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Fish screening assays

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Amphibian metamorphosis assay

Fish sexual development test

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- Fish full lifecycle tests
- Invertebrate lifecycle tests

Fish screening assays

- Hypothalamic-pituitary-gonad (HPG) axis
- OECD 230 21-day fish screening assay
 - Fathead minnow, zebrafish or medaka
 - Vitellogenin and secondary sexual characters
- OECD 229 Short term reproduction assay
 - Fathead minnow only
 - Vitellogenin and secondary sexual characters
 - + fecundity and gonadal histopathology (where appropriate?)
- Adopted September 7 2009



Amphibian metamorphosis assay

- Hypothalamic-pituitary-thyroid (HPT) axis
- *Xenopus laevis* exposed over metamorphosis
- Endpoints
 - mortality, morphological endpoints - whole body length/snout-vent length, hind limb length, wet weight, **developmental stage & histology of thyroid gland**
- Adopted September 7 2009



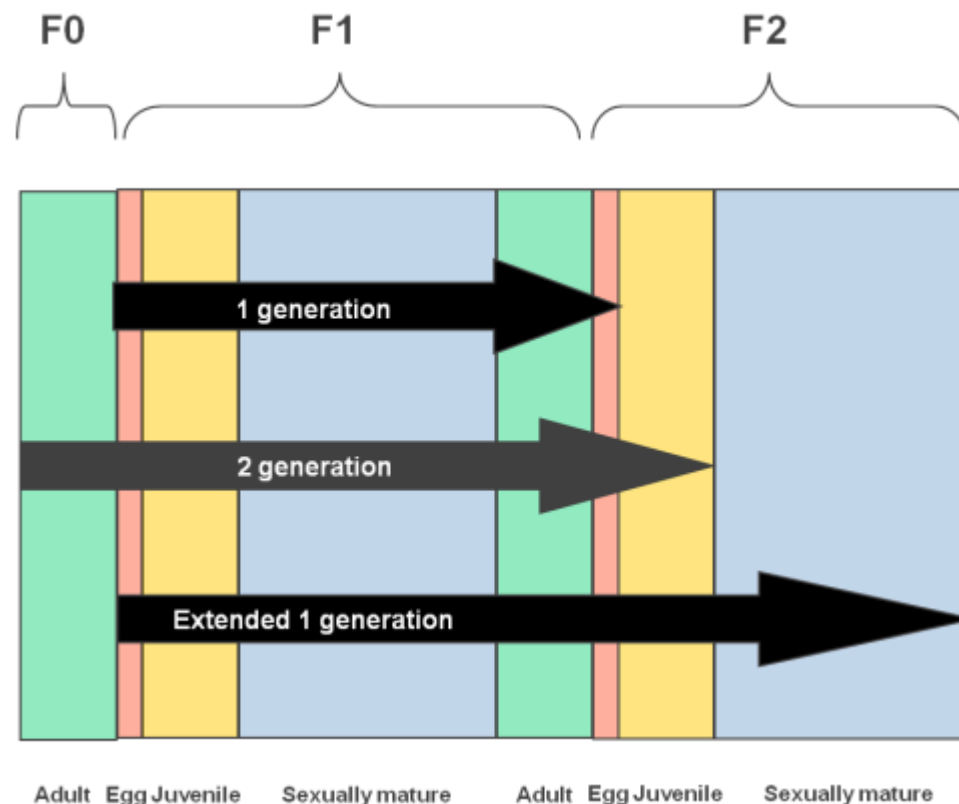
Fish sexual development test

- Hypothalamic-pituitary-gonad (HPG) axis
- Extended early lifestage test
 - Development and growth
 - Histological sex at termination
 - Vitellogenin
- Fathead minnow, zebrafish & medaka
- OECD validation exercise Phase I 2006;
Phase II 2008-2009



Fish full lifecycle tests

- DRP published 2008
 - Range of species
 - Many designs



- Current practise 'bespoke' designs
- US and Japan collaboration medaka 2 generation test

Invertebrates

- Apical tests without mechanistic power
- Copepod lifecycle tests (*Amphiascus*)
- Mysid 2-generation test
- Chironomid lifecycle test
- Mollusc Detailed Review Paper
- Various stages of validation



OECD Workshop

- OECD-EDTA Workshop on OECD Countries Activities Regarding Testing, Assessment and Management of Endocrine Disrupters
- September 22-24th Copenhagen
- Aims
 - to identify the current activities on chemicals with endocrine disrupting properties in member countries
 - to analyze how the Test Guidelines and other tools/data/ information are used for different types and levels of decision making concerning identification, assessment and management of endocrine disrupters
 - develop recommendations for further work and to identify follow-up activities, including further research and co-operation needs

Criteria for identifying endocrine disrupting properties

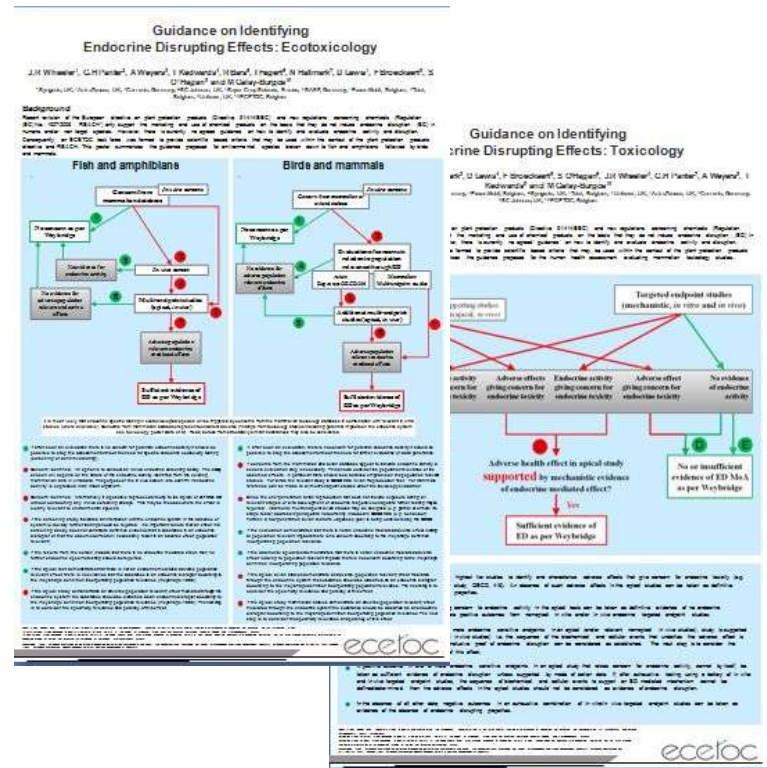
- Cut-off drives need for definition
- European Centre for Ecotoxicology and Toxicology of Chemicals
 - Task force set up June 2008 to develop guidance
 - Toxicology and ecotoxicology
- Report issued June 2009
 - Technical Report No. 106
<http://www.ecetoc.org/technical-reports>
- Tripartite workshop held Barcelona June 29-30th
- Revised guidance in prep

ECETOC guidance

- Common themes were identified from all available ED definitions
 - Weybridge definition employed
- Guidance for the identification of ED was proposed based on data integration from *in vitro* and *in vivo* targeted studies and multi endpoint high tier regulatory (eco)toxicity studies
- Potency consideration was also proposed to discriminate chemicals of high concern from those of lower concern
- Flowcharts developed to aid interpretation

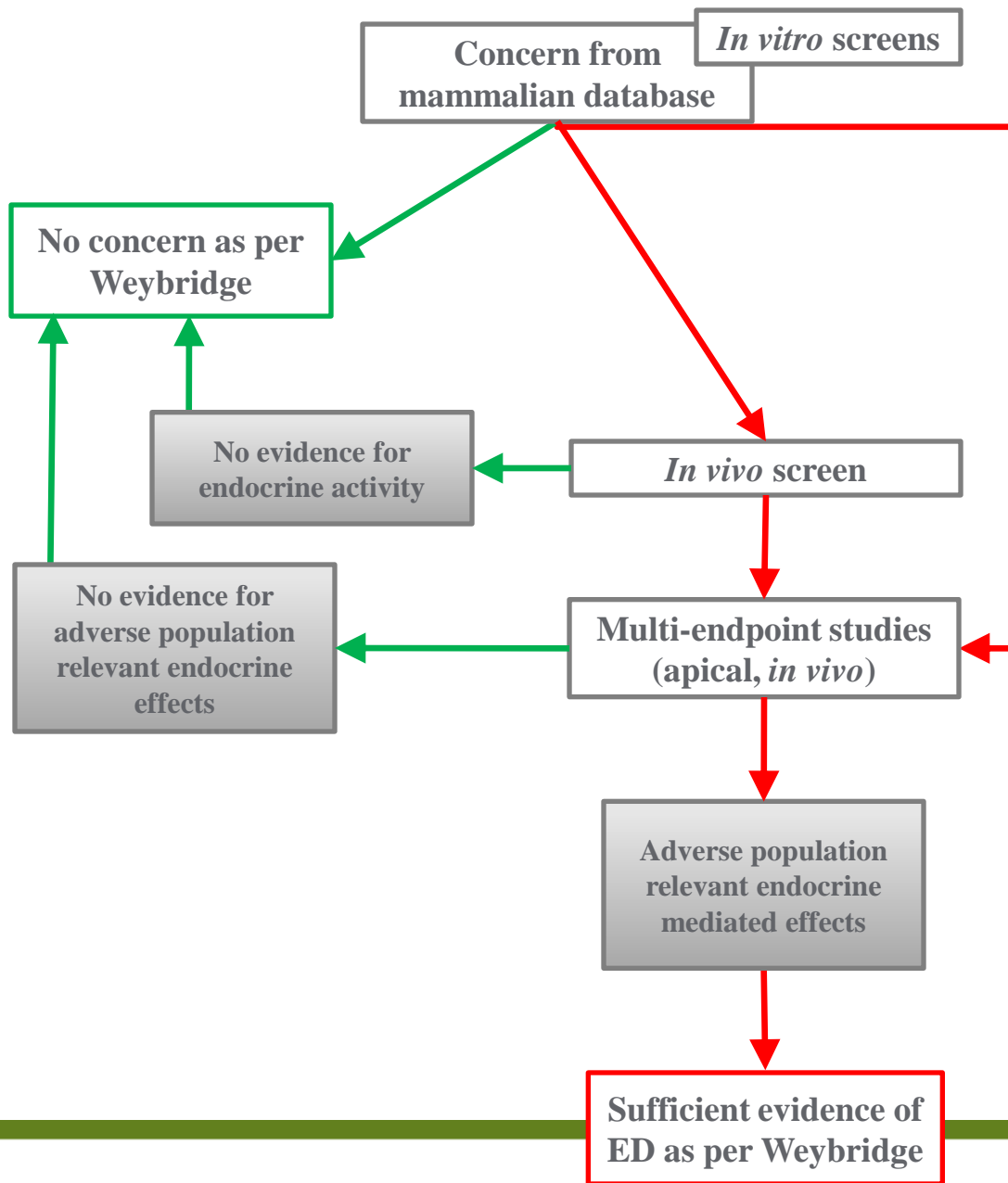
ECETOC guidance

- Scheme for human health assessment
- Scheme for wildlife assessment
 - Fish and amphibia
 - Birds and mammals

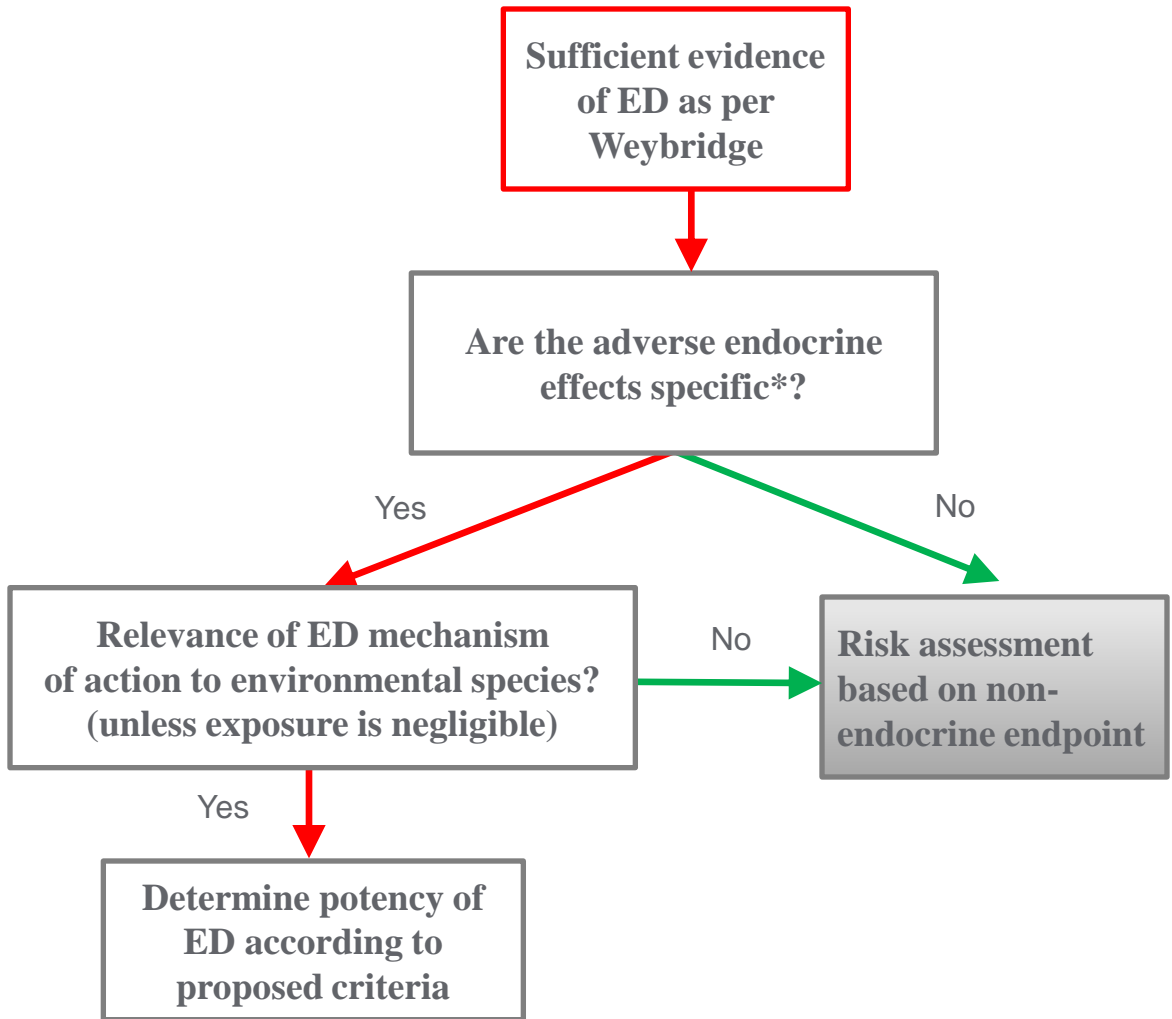


Posters presented at this meeting

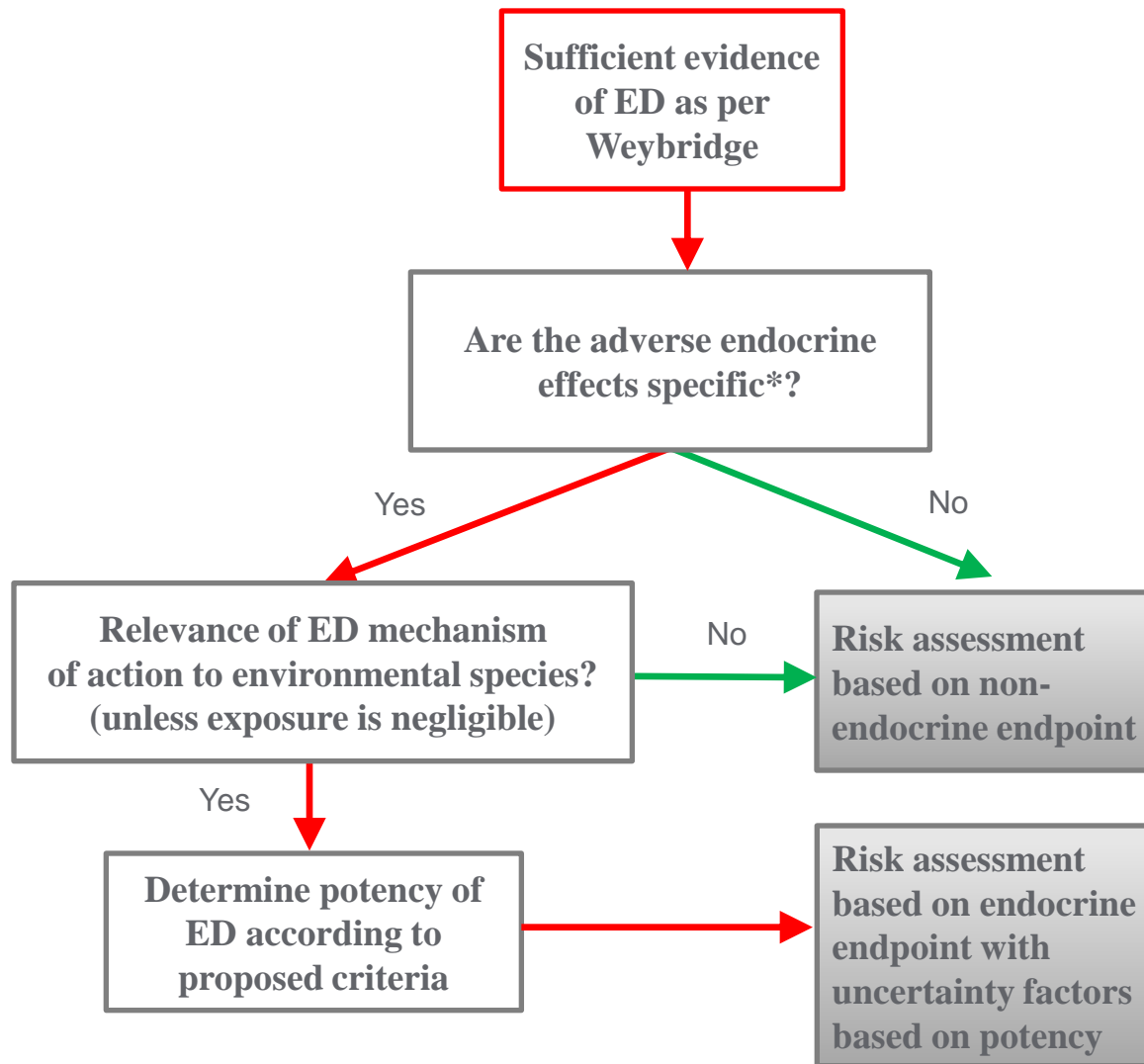
Fish and amphibians



Potency



- Dose / concentration
- Exposure duration
- Taxonomic specificity in relation to the risk assessment
- Type and severity of endocrine mediated effect(s)
- Number of species affected



ECETOC

- ECETOC Task Force has mainly focused on hazard in response to the cut off criterion
- Full evaluation requires risk assessment incorporating:
 - hazard characteristics
 - dose response considerations
 - exposure data
- Risk assessment approach supported by participants at the workshop

Global considerations

- Globally acceptable weight of evidence evaluation is required
- National differences for study requirements
 - US fish short term reproduction design
 - EU confusing which design is acceptable and when
- Harmonisation amongst different legislative instruments for determining endocrine disruption
 - PPPs, biocides and REACH
- US risk based vs EU hazard based approaches

Conclusions

- Revisions to 91/414 include ED as a hazard based cut off
- Definitions of ED are required
 - Proposals are being made (BfR, ECETOC)
- OECD developing screening and definitive test methodologies
- Global and legislative differences in approach