Guidance for the identification of endocrine disruptors

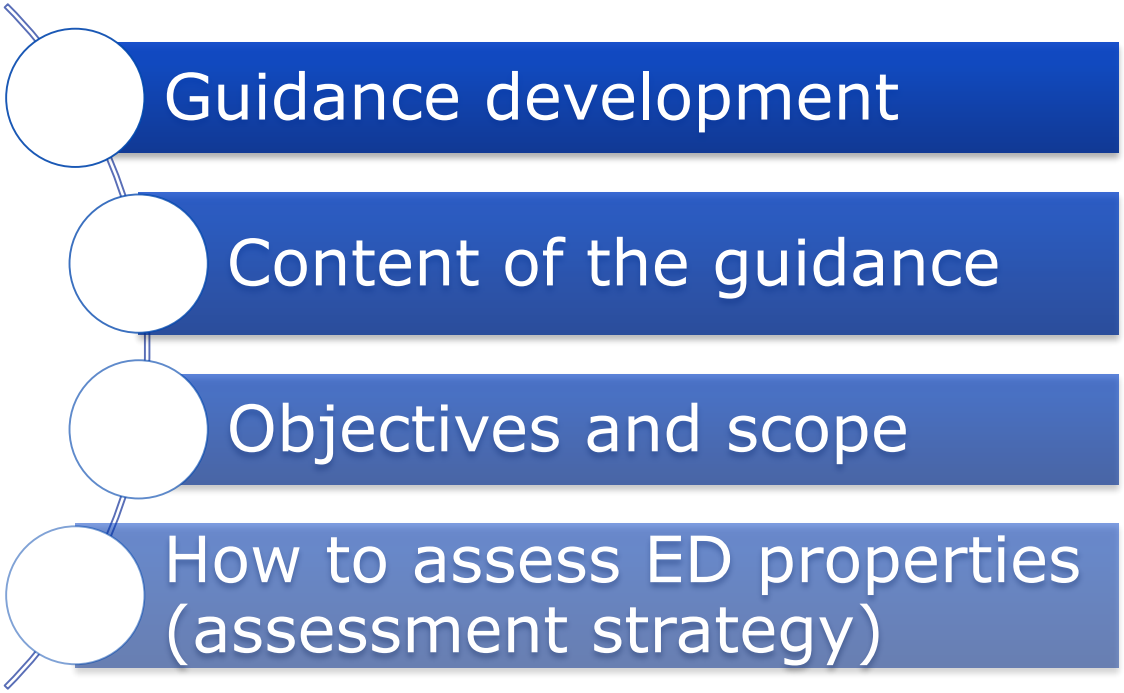
Webinar: Endocrine disruptors and biocides – what you need to know

19 June 2018

Peter Lepper

Risk Management – Classification and Prioritisation

Contents


Guidance development
Content of the guidance
Objectives and scope
How to assess ED properties (assessment strategy)

Guidance drafting and consultation

Drafting by joint team of ECHA/EFSA/JRC scientific staff

Contents of the guidance

1. Introduction
2. Scope of the guidance
3. **Assessment strategy**
4. Information sources for endocrine disruptor identification
5. Recommendations
6. References

Appendices

Objective and scope

Objective of the guidance

- Provide technical guidance for the implementation of the ED criteria in the context of the Biocidal Products and the Plant Protection Products Regulations

  The guidance is for:

  - Applicants
  - Assessors of competent regulatory authorities

Scope of the guidance

- The guidance - like the ED criteria - is based on the WHO/IPCS definition of an endocrine disruptor:

> *"An **endocrine disruptor** 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."*

Scope of the guidance

- Guidance covers endocrine disrupting modes of action caused by estrogen, androgen, thyroid and steroidogenic (EATS) modalities.
  - ⇨ However, available information on potential non-EATS endocrine disrupting modes of action also needs to be followed-up.

- Guidance focuses on ED effects in vertebrates, i.e. mammals (incl. humans), fish, amphibians.

Assessment strategy

Assessment strategy - general features

- **Assessment strategy is set up to use the available data efficiently**
  - ED assessment starts with the data available
    - Dataset must however be compliant with the information requirements of the Biocidal Products Regulation
  - Generation of further data only when necessary
  - Available information on mammals/humans and on non-target organisms is used holistically in the assessment

Assessment strategy
- is based on

- **ED criteria** for Biocides and Plant Protection Products
- **OECD conceptual framework** for testing and assessment of endocrine disruptors
  - Lists the OECD test guidelines and other standardised test methods which can be used to evaluate substances for endocrine disruption
  - Provides guidance on the use of the test methods, but is not a testing strategy
- **OECD guidance document 150** on standardised test guidelines for evaluation of endocrine disruption
  - Helps the interpretation of results for the **parameters (~ effects)** investigated in the assays available for ED testing

Assessment strategy

– is based on grouping of the parameters investigated in available studies

The ED relevant parameters investigated in the available studies are grouped according to the information they provide

(Grouping based on OECD GD 150 & JRC screening methodology to identify potential EDs)

- ***'in vitro* mechanistic'** *parameters;*
- ***'in vivo* mechanistic'** *parameters;*

} *provide information on the mechanism(s) through which a substance is endocrine active*

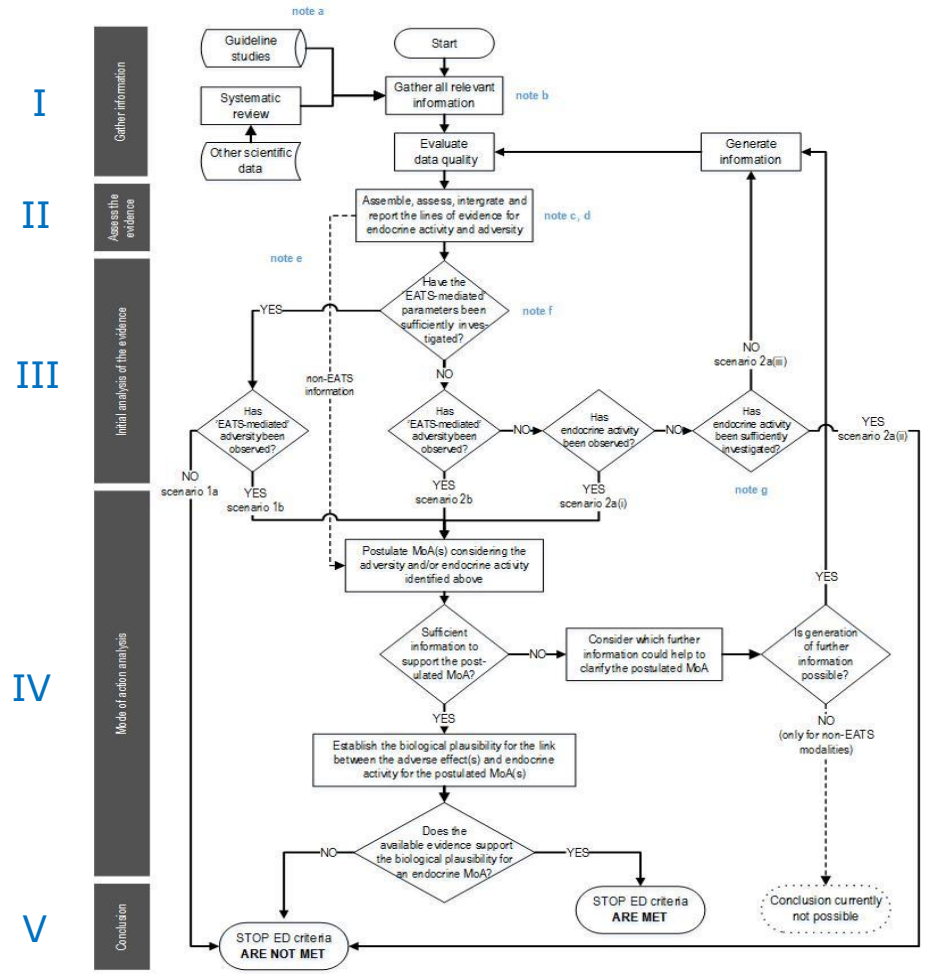
- **'EATS-mediated'** parameters
  Provide information on adverse effects. At the same time, due to the nature of the effect and the existing knowledge, they are also considered indicative of an EATS endocrine mechanism and thus (in the absence of other explanations) provide in vivo explanation of the endocrine mechanism resulting in the adverse effect.

- **'Sensitive to, but not diagnostic of, EATS'** parameters
  Provide information on adverse effects. However, due to the nature of the effect and the existing knowledge, these effects cannot be considered (exclusively) diagnostic of underlying EATS related endocrine activity. Nevertheless, in the absence of more diagnostic (i.e. EATS-mediated) parameters, these effects might provide indications of a potential endocrine mode of action that might warrant further investigation.

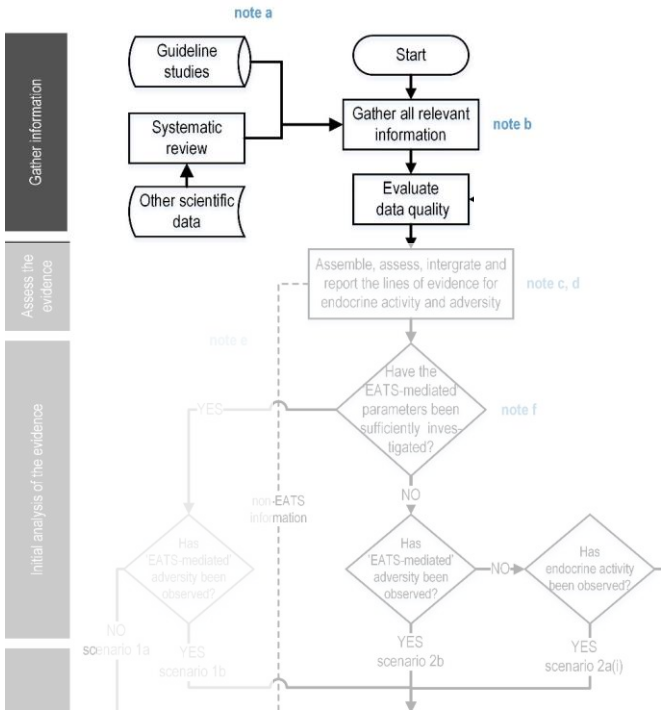
Assessment strategy

Flowchart illustrating the steps of the ED assessment strategy

(Figure 1 of the guidance document)



I) Gather all relevant information



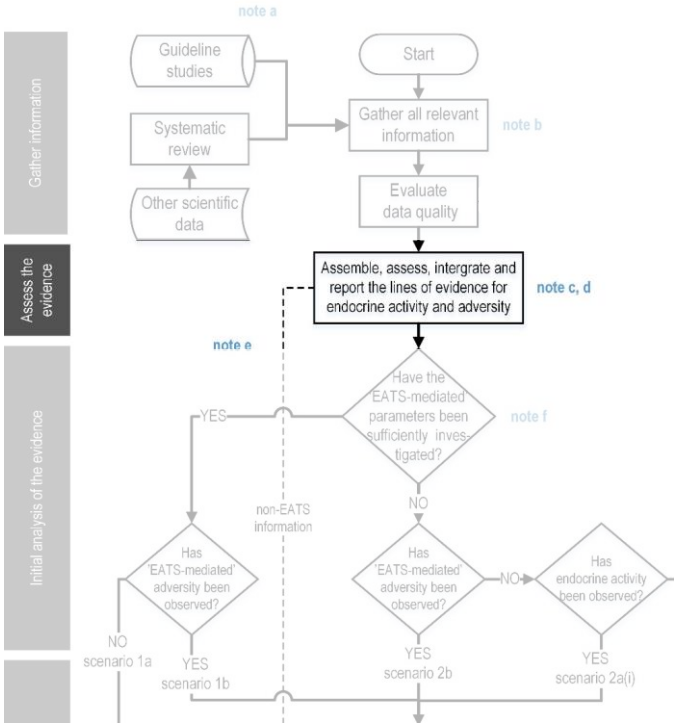
All relevant information must be considered:

- guideline studies
- other scientific data selected through systematic review

➢ Evaluation of data quality according to the provisions of Biocidal Products Regulation

➢ From valid studies assign all parameters relevant for ED assessment to the groups:
  - *In vivo* mechanistic
  - *In vitro* mechanistic
  - EATS-mediated
  - Sensitive to, but not diagnostic of, EATS

II) Assemble, assess and integrate the lines of evidence



Assemble all available data and integrate it into lines of evidence based on the grouping:

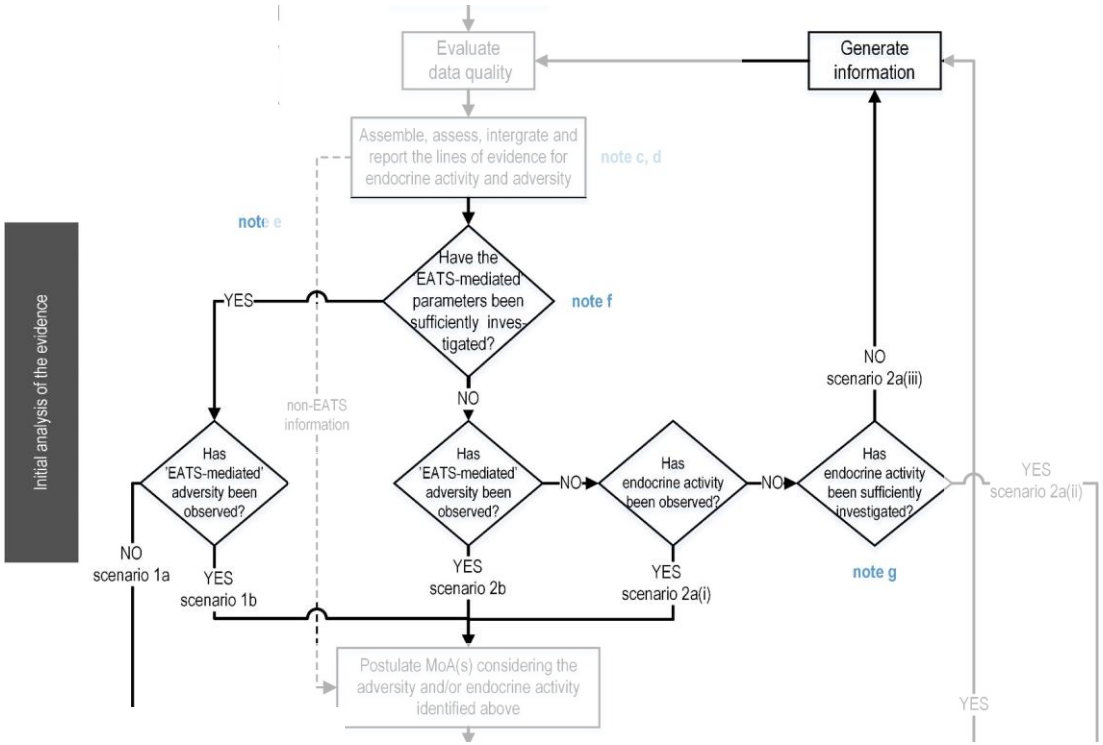
- **Lines of evidence for adversity** from:
  - 'EATS mediated' parameters
  - 'sensitive to, but not diagnostic of, EATS' parameters
- **Lines of evidence for endocrine activity** from:
  - '*in vitro* mechanistic' parameters
  - '*in vivo* mechanistic' parameters
  - 'EATS mediated'- parameters

Example of lines of evidence

Table 2: Example showing how to assemble, integrate and assess the lines of evidence for thyroid disruption in mammals

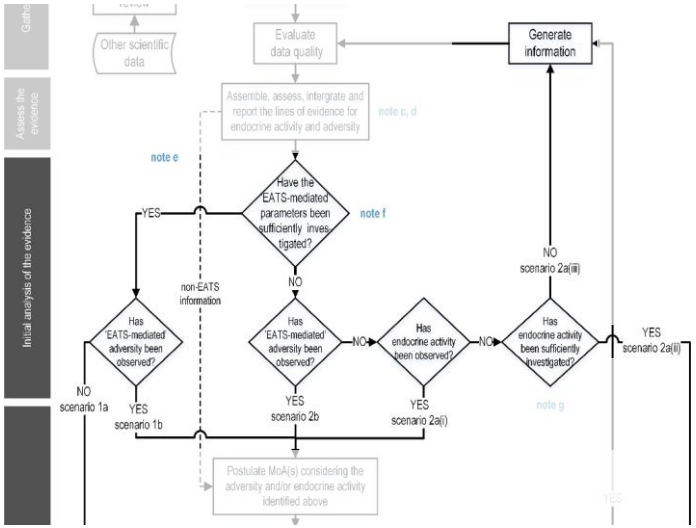
	Grouping	Line(s) of evidence	Species	Exposure Weeks	Route of exposure	Effect dose mg/kg/day	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
Integrated line of evidence for endocrine activity	*In silico* prediction	(Q)SAR prediction					Predicted to Inhibit iodine transport	Supporting evidence	Overall positive evidence for endocrine activity	Thyroid
	In vivo mechanistic	Hormonal changes T3, T4	Dog	26	Oral	13	dose dependent decrease	Hormone changes observed in three species in a dose related manner		Thyroid
			Hamster	78	Oral	15	no effect; highest dose tested 15			
			Rat	4	Oral	5	dose dependent decrease			
			Rat	4	Inhalation	0.32	dose dependent decrease			
			Rabbit	2	Oral	75	dose dependent decrease			
Integrated line of evidence for adversity	EATS-mediated parameter	Hind limb length	Frog	3	Water	1.75	dose dependent decrease	Sufficient: changes observed in a dose dependent manner	Overall positive evidence for adversity	Thyroid
		Thyroid (histopathology)	Frog		Water	1.75	dose dependent increase	Sufficient: changes observed in a dose dependent manner		
		Thyroid (histopathology)	Lizard	4	intraperitoneal injections	5	Changes in epithelium height of the follicular cells at all the tested doses	Supportive (non-standard species and study design) evidence of changes in histopathology in a dose		

III) Initial analysis of the evidence



Initial analysis of the evidence

5 questions – 3 possible outcomes



Outcomes

1)Conclude 'ED criteria not met' if:

- 'EATS mediated' parameters **sufficiently investigated** and no EATS mediated adversity observed

  Or

- Endocrine activity sufficiently investigated and no endocrine activity observed (and also no EATS mediated adversity)

2)Move to MoA analysis if:

- EATS mediated adversity observed

  Or

- Endocrine activity observed

3)Generate information if:

- No EATS mediated adversity and no endocrine activity observed but endocrine activity not sufficiently investigated

When are 'EATS-mediated' parameters sufficiently investigated? (for humans & mammals)

- **Sufficient that the data requirements of the BP Regulation are fulfilled and studies carried out in accordance with actual protocols**

- **'EAS-mediated'** parameters
  - All 'EAS-mediated' parameters foreseen to be investigated in a two generation reproductive toxicity study (**OECD TG 416**) measured

  or

  - All 'EAS-mediated' parameters foreseen to be investigated in an extended one generation reproductive toxicity study (**OECD FTG 443;** EOGRTS) measured

- **'T-mediated'** parameters
  - Thyroid parameters foreseen to be investigated in the required standard studies for repeated dose toxicity, reproductive toxicity and carcinogenicity.

When is 'endocrine activity' sufficiently investigated? (for humans & mammals)

- **ONLY required if EATS-mediated parameters not sufficiently investigated**

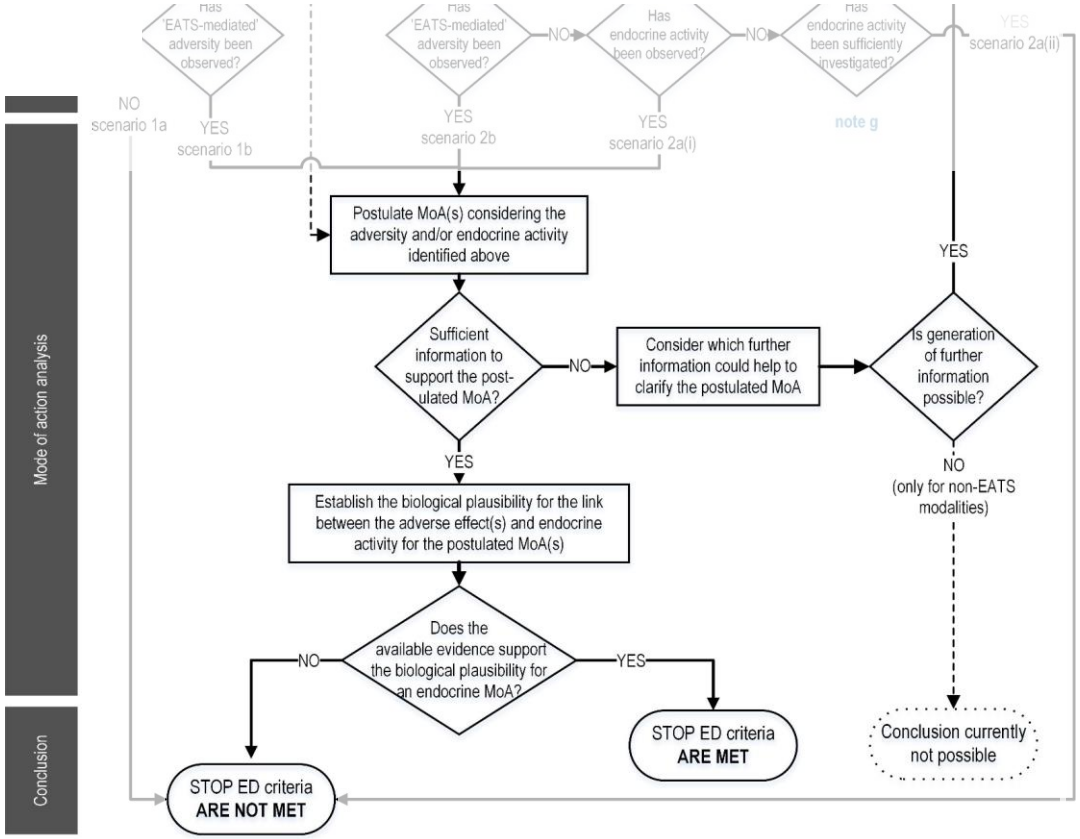
- **E-modality** – Output data from the ToxCast ER Bioactivity Model or 'Uterotrophic bioassay in rodents' (OECD test guideline 440).
- **A-modality** – 'Hershberger bioassay in rats' (OECD test guideline 441).
- **T-modality** – Thyroid parameters foreseen to be investigated in the required standard studies for repeated dose toxicity, reproductive toxicity and carcinogenicity (same as for T-mediated parameters).
- **S-modality** – 'H295R steroidogenesis assay' (OECD TG 456) and the 'aromatase assay (human recombinant)' (OPPTS 890.1200) carried out.
  The results of these 2 assays should be considered together with the results of the E- and A-modalities to conclude on the absence of S-related endocrine activity.

No EATS-mediated adversity or endocrine activity observed but 'EATS-mediated' and 'endocrine activity' parameters not sufficiently investigated

- **Generation of further data required**
  - Either
    Assessment of missing EATS-mediated parameters until 'sufficiently investigated'
  - Or
    Assessment of endocrine activity until 'sufficiently investigated'

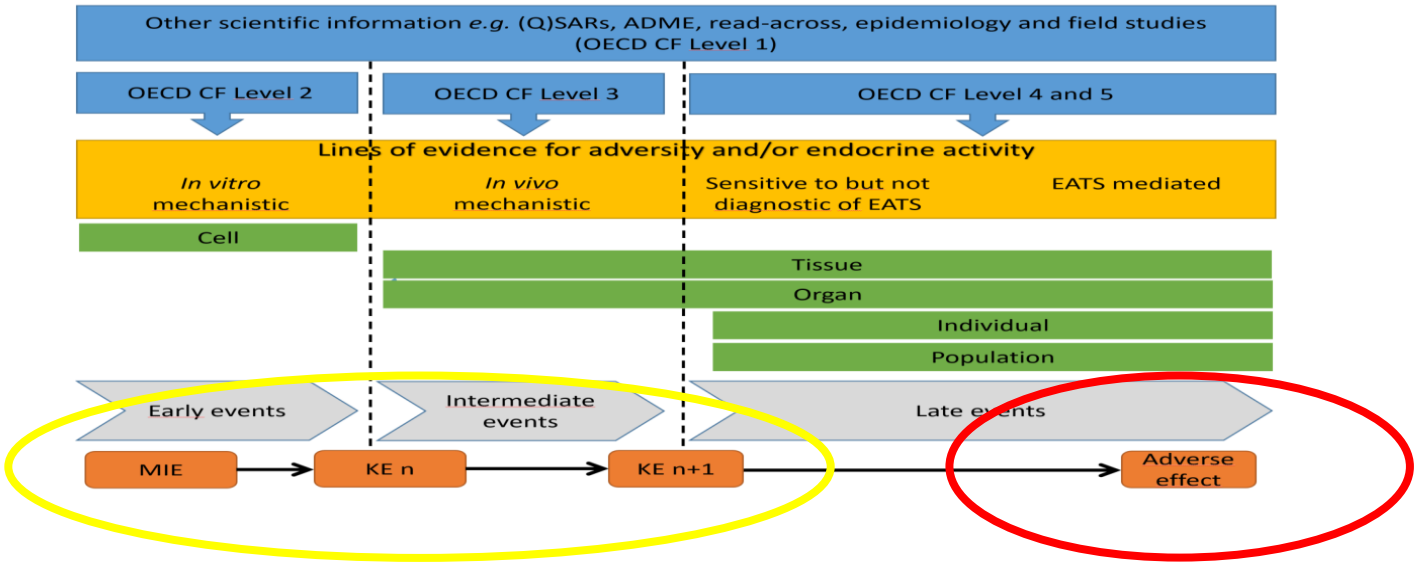
- For a conclusion that ED-criteria are not met a 'sufficiently investigated' dataset with negative results is necessary either on EATS-mediated parameters or on EATS endocrine activity

IV) Mode of action analysis



Mode of action analysis (MoA)

- A MoA is a series of biological events that result in a specific adverse effect
  - For an endocrine disrupting MoA at least one of the biological events involves element(s) of the endocrine system



- **Mode of action analysis is required if 'EATS-mediated' adverse effect(s) or endocrine activity (or both) have been observed**

Mode of action analysis (MoA)

- First step of the MoA analysis is to postulate one or more MoA(s) using the available lines of evidence
- Consider whether the information of the lines of evidence is sufficient to postulate the MoA(s)
  1. If the information is sufficient to support the postulated MoA, it is possible to assess whether there is a biologically plausible link between endocrine activity and adverse effect(s) and to conclude whether the ED criteria are met
  2. If the available information is not sufficient, further information is needed to demonstrate the postulated MoA(s)
- It may not be necessary to establish the whole sequence and relationship of events leading to adverse effect(s) to conclude on the biological plausibility of the link between endocrine activity and adverse effect
  - Existing knowledge on endocrinology / toxicology may be sufficient to conclude on the biological plausibility
    (e.g. if the MoA is mainly established and empirically supported on the basis of EATS-mediated parameters)

Example of a postulated MoA

Summary of the hypothesis: The substance increases serum estradiol in a dose-dependent manner. This results in continuous estrogen receptor 1 activation in the uterus. The increased estrogen signalling ultimately results in cancer.

	Brief description of key event (KE)	**Supporting evidence**
Molecular initiating event (MIE)	Inhibition of androgen synthesis (postulated MIE)	None (no data provided, but hypothesised based on current knowledge and former experience with chemicals)
KE 1	Increased serum estradiol	Increased serum estradiol (OECD TG 407)
KE 2	Uterine hypertrophy	Increased uterine weight (OECD TG 407 and 408)
KE 3	Uterine hyperplasia	Histopathology (OECD TG 408 and 453)
Adverse effect (AE)	Uterine neoplasia	Histopathology (OECD TG 453)

V) Conclusion whether the substance assessed meets the ED criteria

- Where, based on a sufficient dataset, no 'EATS-mediated' adversity is observed, or where endocrine activity is found negative, the mode of action analysis can be by-passed and it can be concluded that the ED criteria are not met.
- Where a mode of action is based on 'EATS-mediated' adversity, the ED criteria are considered met, *unless …*
- Where a mode of action is based on 'sensitive to but not diagnostic of EATS' adversity and the weight of evidence supports the biological plausibility of the link between adverse effects and endocrine activity, the ED criteria are considered met, *unless …*
- **…unless** an alternative non-endocrine mode of action is demonstrated and in a comparative analysis found to be the most likely explanation.

Take home

- The Guidance document provides both applicants and assessors from regulatory bodies with guidance on carrying out the ED assessment or evaluating it
- The assessment strategy is set up to use the available data efficiently
- Information as required for the core data set of the information requirements for biocidal active substances may be sufficient to carry out the ED assessment
- Generation of further data only when necessary
- Existing knowledge on EATS-mediated adverse effects may be sufficient to carry out the MoA analysis and conclude on the biological plausibility of a link between endocrine activity and observed adverse effect(s)

Thank you

Subscribe to our news at
echa.europa.eu/subscribe

Follow us on Twitter
@EU_ECHA

Follow us on Facebook
Facebook.com/EUECHA