Gesellschaft für Toxikologie

GT Advanced Course 2024
„EDs: Status and Testing Strategies in Different Legislations Considering the 3Rs“
of the Working Groups Regulatory Toxicology and 3R/Alternative Methods

Login

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GT Advanced Course 2024
„EDs: Status and Testing Strategies in Different Legislations Considering the 3Rs“

09:00	Opening remarks: Introduction with a focus on the regulatory background	Michael Werner, AK RegTox
Session 1:	**Overview of available testing methods and testing strategies with a focus on non-animal methods**	Moderation: Tina Hofmaier, AGES
09:15	Available/accepted methods/strategies	Stefanie Melching-Kolmuss, BASF (confirmed)
09:45	Endpoint specific methods in development and to come (EU and non-EU)	Anne Gourmelon, OECD principle administrator (confirmed, online)
10:15	The EFSA database on pesticides endocrine disrupting properties and the impact of the assays measuring the endocrine activity	Andrea Terron/Martina Panzarea, EFSA EDWG (confirmed, online)
	10:45 Coffee break	
Session 2:	**Regulatory aspects in ED assessment and status during evaluation procedures**	Moderation: Philip Marx-Stölting, BfR
11:05	Regulatory approaches PPP/Biocides and experiences gathered so far	Vera Ritz, BfR (confirmed)
11:35	Regulatory approaches under REACH and other regulations (cosmetics, medical devices, food contact materials) - An overview of experiences	Michaela Moors-Frericks, knoell Germany (confirmed)
12:05	CLP regulation and C&L for ED effects – State of the play, challenges, implementation	Nicolaj Heuer, BfC (confirmed)
	12:35 Coffee break	
12:55	Panel discussion – Challenges in the Establishment, Validation and Acceptance of ED Testing Methods from the Scientific and Regulatory Point of View	All Panellists and moderators
13:45	End of Advanced Course	

Purpose of the Advanced Course on ED

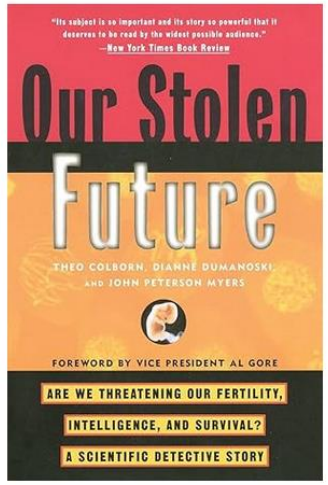
- Gain an overview of the *in vitro/in vivo* testing methods for the investigation of potential ED properties
- Discuss the challenges in testing considering that animal testing should be the last resort
- Touch upon the new hazard classes and categories for the endpoint “ED” under the CLP regulation
- Discuss the impact of the identification of a substance as a potential ED from the regulatory perspective

Assessment of potential ED properties

❖ **„Historical“ starting point and awareness on effects on hormone system:**

- **Potential ED properties and regulatory actions were and are under discussions in different countries** for a very long time **starting with findings of sexual transformation in wildlife animals caused by exposure towards substances via the environment.**

- **1997: “Our stolen future” by Theo Colborn, Dianne Dumanoski and John Peterson Myers**



- ***examines the ways that certain synthetic chemicals interfere with hormonal messages involved in the control of growth and development, especially in the fetus.***
- ***explores the scientific discovery of endocrine disruption. The investigation begins with wildlife, as it was in animals that the first hints of widespread endocrine disruption appeared.***
- ***summarizes a series of well-studied examples where people have been affected by endocrine disrupting chemicals, most notably the synthetic hormone diethylstilbestrol (DES).***

- **US EPA ED screening program within the scope of the “EDSP21” (**molecular based *in vitro* high-throughput screening assays to screen chemicals for their potential estrogen, androgen or thyroid (E, A, or T) bioactivity)**.**

Assessment of potential ED properties

❖ **Scientific and regulatory starting point for ED assessment of (active) substances in the EU:**

- **Criteria for the determination of ED properties** were **not available yet by the time of application of the original versions of the** the Biocidal Products Regulation (BPR, Regulation (EU) No 528/2012) and the Plant Protection Products Regulation (PPPR, Regulation (EC) No 1107/2009).
  - **ED-specific requirements in both regulations:** No later than **13 December (BPR)** and **14 December (PPPR) 2013**, **measures for the determination of endocrine-disrupting properties shall be adopted** in accordance with the respective applicabe regulatory procedures.

  - **Pending scientific criteria** for ED the **interim criteria** applied under the BPR and PPPR:
    → C&L acc. to CLP (Regulation (EC) No. 1272/2008) with Carc. Cat. 2 and Reprotox. Cat. 2 **shall be** considered as ED.
    → C&L acc. to CLP (Regulation (EC) no. 1272/2008) Reprotox. Cat. 2 and specific effects on endocrine organs **may be** considered as ED.

Scientific criteria for assessment of potential ED properties

- **Development/implementation of the scientific criteria for ED properties of (active) substances within the meaning of the BPR and PPPR:**
  - **Active substances used in biocides:** Commission Delegated Regulation (EU) No. 2017/2100 (applied as of 07 June 2018)

17.11.2017 EN Official Journal of the European Union L 301/1

II

(Non-legislative acts)

REGULATIONS

COMMISSION DELEGATED REGULATION (EU) 2017/2100

of 4 September 2017

setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to Regulation (EU) No 528/2012 of the European Parliament and Council

(Text with EEA relevance)

  - **Active substances used in plant protection products:** Commission Regulation (EU) No. 2018/605 (applied as of 10 November 2018)

20.4.2018 EN Official Journal of the European Union L 101/33

COMMISSION REGULATION (EU) 2018/605

of 19 April 2018

amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties

(Text with EEA relevance)

Scientific criteria on ED properties – Main elements

❖ According to Regulations (EU) No. 2017/2100 and 2018/605 as well as the ED GD **a substance shall be considered as having ED properties if it meets all of the following criteria:**

➢ **3 Key elements to be fulfilled for an ED substance both for humans and non-target organisms:**

- **Adverse effect in an intact organism or its progeny is observed** (*change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences)* ***→ WHO definition of an ED!***

and

- **Endocrine mode of action** is identified (*altering the function(s) of the endocrine system*)

and

- **Adverse effect is a consequence of the endocrine mode of action**

All criteria are to be met!

Key message:

“Establishment of a “plausible biological link” between adverse effect and a proven endocrine activity”

Scientific criteria for assessment of potential ED properties

Regulation (EU) No. 2017/2100 + 2018/605
Annex: ED criteria for BPs + PPPs

→ **Biocides the first legislation to apply ED criteria!**

Section A — Endocrine-disrupting properties with respect to humans

Section B — Endocrine-disrupting properties with respect to non-target organisms → focus is on differentiation between taxonomic groups and (sub)population level

→ **Derogation for ED effects on non-target organisms not included for PPPs!**

Main elements:

- Gathering of all relevant scientific ED-related data (*in vivo*, *in vitro*, *in silico)*
- **(Re-)Assessment** of all **relevant scientific data with a particular focus on ED-related endpoint/effects** applying a weight-of-the-evidence approach and considering:
  - Positive/negative results
  - Relevance of study design, quality, consistency of data
  - Exposure route and toxicokinetics
  - Limit dose/MTD (maximum tolerated dose) considerations

Important: Adverse effects of non-specific consequences of toxicity which are **not to be considered/not relevant** for the determination of **potential ED properties!**

All criteria are to be met to conclude on presence or absence of ED properties!

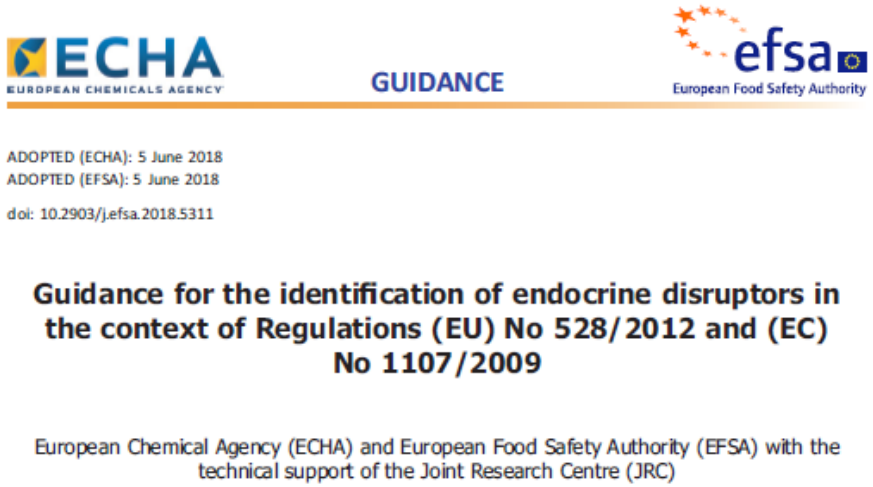
ECHA/EFSA ED Guidance Document

❖ Scientific criteria:

- **Define only a (regulatory) basis and roughly the steps to be taken towards the assessment of available data** of active substances for the determination of endocrine disrupting properties **for humans and non-target organisms** within the meaning of the BPR and PPPR.
- **Scientific criteria provide no tools/guidance/guidelines for tiered testing approaches and how to perform the ED assessment.**

❖ ECHA/EFSA ED GD towards the identification of ED properties:

- **Performance of science-based ED assessments** started with the application of the „ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009”on 07 June 2018.


ECHA
EUROPEAN CHEMICALS AGENCY
GUIDANCE
efsa
European Food Safety Authority

ADOPTED (ECHA): 5 June 2018
ADOPTED (EFSA): 5 June 2018

doi: 10.2903/j.efsa.2018.5311

Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

European Chemical Agency (ECHA) and European Food Safety Authority (EFSA) with the technical support of the Joint Research Centre (JRC)

ECHA/EFSA ED Guidance Document (GD)

❖ Focus of the ECHA/EFSA ED GD

- ED scientific guidance is dedicated to biocides and plant protection products but is, in principle, **applicable to an ED assessment of any substance** as well irrespective of the regulatory context

- **All stages for ED assessment** of a substance follow a **strictly hazard-based approach → no considerations on potency, exposure/risk!**

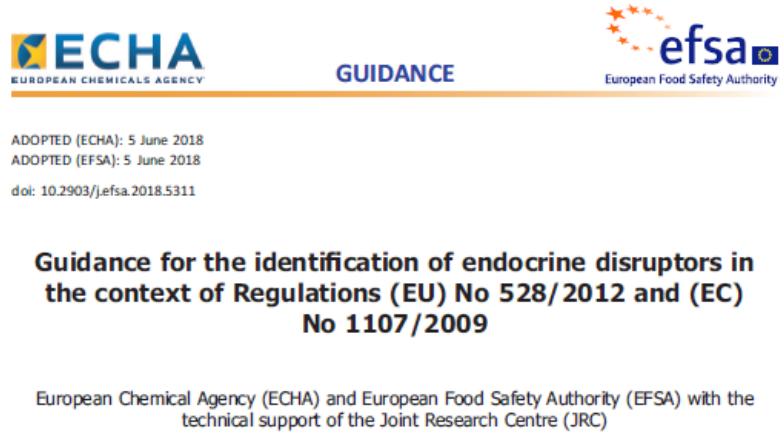
- **ED modalities addressed in the ED GD:**

  - **ED GD addresses only "EATS" modalities (estrogenic-, androgenic-, thyroid- and steroidogenic-related pathways) with a focus on ED effects in vertebrates**, i.e. mammals (humans), fish, amphibians

    → **EATS modalities are best understood from the mechanistic point of view!**

    → **standardised *in vitro/in vivo* test methods and guidelines are available** on these endpoints with **broad scientific agreement on the interpretation of the effects** observed on the investigated parameters

  - Note: **non-EATS pathways are outside the scope of the ED GD** but should also be addressed in the context of the **evaluation of modes of action!**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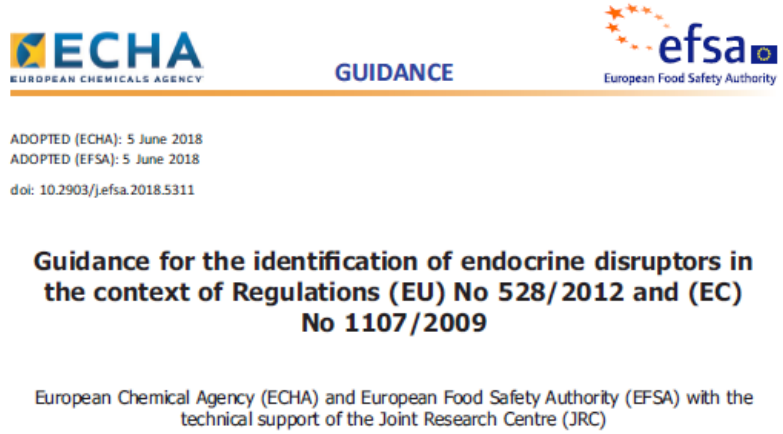
❖ Assessment and testing strategy towards the identification of EDs is based on

- **OECD GD 150:** Listing tests/studies and parameters relevant for the investigation of ED properties including their interpretation
- "**OECD Conceptual Framework (CF)** for Testing and Assessment of EDs"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❖ Grouping and interpretation of study types of the OCED CF

- ***In vitro* mechanistic:** *In vitro* parameters of OECD CF level 2 providing information on a potential endocrine mechanism (i.e. receptor binding/activation, interaction **with hormone production)**
- ***In vivo* mechanistic:** *In vivo* parameters of OECD CF level 3 providing information on usually non-adverse endocrine activity (e.g. hormone measurements *in vivo*, Hershberger assay)
- **EATS-mediated:** *In vivo* parameters contributing to the evaluation of adversity → indicative of an EATS mode of action with underlying *in vivo* mechanistic information (e.g. organ weights/(histo)pathology of endocrine organs)
- **Sensitive to, but not diagnostic of, EATS:** *In vivo* parameters from OECD CF levels 3-5 → contributing to the evaluation of adversity → **not sufficiently indicative on their own of any one of the EATS modalities**

ECHA/EFSA ED Guidance Document (GD)

❖ ECHA/EFSA ED GD – 5 stages towards assessment of ED properties

1. **Gather all relevant information/parameters relevant for ED assessment**
   - ➢ *In vivo/in vitro* mechanistic data (**Levels 2 + 3 of OECD CF,** eg. ToxCast data)
   - ➢ EATS-mediated parameters (**Levels 4 + 5 of OECD CF**)
   - ➢ Sensitive to, but not diagnostic of, EATS parameters (**Levels 3 – 5 of OECD CF**)

2. **Assemble, assess and integrate the lines of evidence**
   - ➢ Assessment of adverse effects with a view to ED relevance ("**EATS-mediated parameters**" and "**sensitive to, but not diagnostic of, EATS parameters**") and of available **information on ED activity** (*in vivo/in vitro* mechanistic data, EATS parameters)

3. **Initial analysis of the evidence**
   - ➢ Judgement if **EATS-mediated parameters/ED activity are sufficiently investigated** and establishment of a potential biological plausible link between (**EATS-mediated) adversity** and **potential ED activity**

4. **Mode of action analysis**
   - ➢ if EATS-mediated adversity **and/o**r ED activity is observed → in depth **analysis of the mode of action** and of "cascade" **MIE → KE_{1-n} → AO***
     ***MIE: molecular initiating event; KE: key event; AO: Adverse outcome**

5. **Conclusion whether the substance meets the ED criteria**
   - ➢ Conclusion on positive/negative ED potential **or** conclusion not possible → **generation of further data *in vitro* and/or *in vitro***

ECHA/EFSA ED Guidance Document (GD)

ECHA
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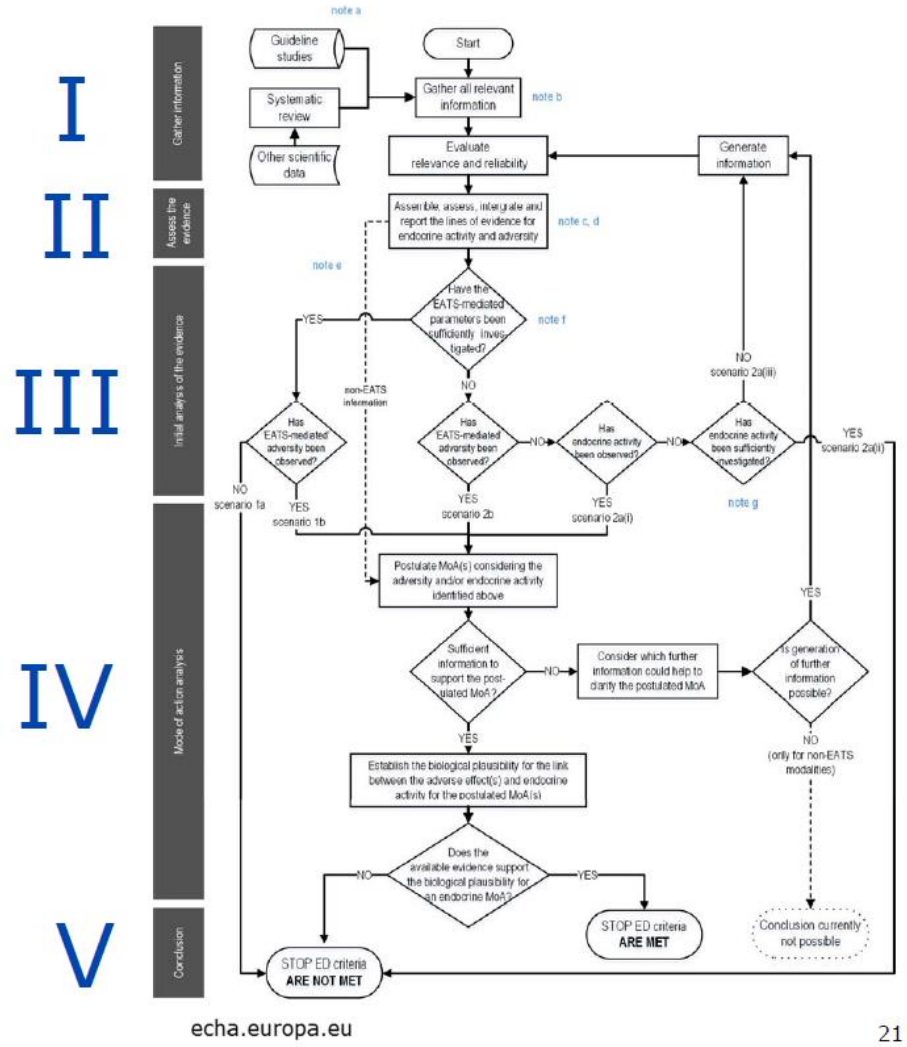
Flowchart illustrating the ED assessment strategy



Flow chart on the 5 stages towards the assessment of ED properties

Assessment of potential ED properties

❖ **ED Properties: Regulatory implications on the approval of biocidal active substances**

- ➢ **Positive outcome in ED assessment** only **another „tag“ for C&L** of a substance**?**
- ➢ **No (major) implication on use of a substance/product containing this substance?**

Section A of Regulation (EU) 2017/2100: Humans	Section B of Regulation (EU) 2017/2100: Non-target organisms
○ ***Exclusion criteria acc. to Art. 5(1) of the BPR apply if:*** a) substance is meeting the ED criteria according to Section A of the Annex to Regulation (EU) 2017/2100 b) substance identified in accordance with Article 57(f) and 59(1) of the REACH Regulation (EC) No 1907/2006 as having ED properties. **Consequence: Phase out of active substance** if **derogations acc. to Article 5(2) of the BPR are not applicable.**	○ ***Substance is a candidate for substitution acc. to Art. 10(1) of the BPR if:*** a) substance is meeting the ED criteria according to Section B of the Annex to Regulation (EU) 2017/2100 b) substance has an intended mode of action that consists of controlling target organisms via their endocrine system(s) **Consequence: Comparative risk assessment will be required and approval and each renewal only for a period not exceeding seven years.**

ED Assessment and the principle of "One Substance One Assessment (OSOA)

❖ Objective of OSOA

➢ Concordant hazard assessment for one substance

- Independent of (horizontal) legislation and evaluating regulatory body
- Human health data requirements independent of intended applications/exposure scenarios across regulatory areas and industrial sectors

➢ Requirements

- Common (and complete!) toxicological data set available and accessible for one substance
- Application of agreed common principles in hazard assessment, i.e. **same understanding in the interpretation of observed findings as adverse vs. non-adverse effects vs. adaptive responses!**

➢ Challenges in real-world scenarios

- Due to differences in data requirements, the type, extent and availability of toxicological data packages for one substance are very heterogenous

ED Assessment and the principle of "One Substance One Assessment (OSOA)

❖ Comparison data requirements of horizontal legislations

Biocides (Regulation (EU) No 528/2012) Agrochemicals (Regulation (EC) No 1107/2009)	REACH (Regulation (EC) No 1907/2006)
• **Complete toxicological data package** required for active substances used in biocidal and plant protection products **independent of intended applications, product types and target organisms** ↓ • Toxicological data packages for active susbstance approval need to enable – **robust assessment of the hazard profile** including potential **endocrine disrupting properties** – **derivation of reliable reference values** for different **exposure durations** and **user categories** ↓	• **Data requirements dependent on tonnage** • **At lower tonnage bands** (1-10t/a, Annex VII of REACH), **only** a **limited hazard assessment** is **possible** • **„higher tier" endpoints** to be generated/submitted **only at tonnage bands > 100t/a (Annex IX and X of REACH)** ↓ • **Consequences of tiered/tonnage-based data requirements:** – **Comprehensive and reliable hazard assessment under REACH hampered due to tonnage-based approach** – **Deduction of reliable reference values** only **possible at higher tonnage bands** or **additional assessment/uncertainty factors** necessary ↓
OSOA-compliant hazard assessment	**and harmonized POD deduction unlikely!**

Thank you very much for your attention