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Assembling and evaluating lines of evidence and definition of adversity: ecotoxicology

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Assembling the lines of evidence: non target organisms

- For non-target organisms, separate lines of evidence for adversity could be assembled for the different **species/taxa**.
  - data on **fish** could be used for assembling lines of evidence for **E, A and S modalities**
  - data on **amphibians** could be used for assembling lines of evidence for the **T modality**.

- In some cases, data on amphibians may also inform about the E, A and S modalities (i.e. OECD TG 241)

- The lines of evidence for adversity on non-target organisms could be built by considering e.g. the **reproduction** in the case of E, A and S modalities and/or the **development/growth** for the T modality.

- Data on **other taxa** (e.g. birds) can, on a case-by-case basis, be considered as supplementary information.

Assessing the lines of evidence

- The assessment of the lines of evidence is based on the available **empirical support** (dose-response, temporal concordance, consistency among studies and species and repeatability) and **expert judgement**.

- In the case of the lines of evidence for adversity related to **non-target organisms**, the empirical support will be mainly based on the evaluation of the dose-response relationship (data frequently available for only one species, normally less studies available than in the case of mammals)

Integration of the lines of evidence for adversity

- Once assembled, the available lines of evidence should be integrated for the assessment of adversity for each modality
- Additional information, e.g. on systemic toxicity or other target organ effects is considered at this point
- The assessment of the integrated lines of evidence includes an evaluation of whether the data set is sufficient to support robust conclusion on adversity

Population relevance

- Before deciding whether the available evidence are sufficient for the definition of adversity, the population relevance of the effects should be considered, in accordance with the Commission Regulation N° 2018/605.
  - Effects on growth, development and reproduction are generally considered relevant
  - For mammals, thyroid hystopath findings in isolation (in the absence of development and reproductive effects) are not considered relevant
  - For amphibians, thyroid histopath findings are relevant when observed together with effects on development

Example 1

Test type (species)	Effect classification	Line(s) of evidence	Exposure (weeks)	Observed effects	Assessment of each LoE	Assessment of the integrated LoE	Modality
FSTRA-OECD TG 229 *(Pimephales promelas)*	EATS mediated	Male SSC in females and males	3	No effects observed up to the highest tested concentration	No effects observed up to the highest tested concentration	Overall, the available evidence is considered insufficient for the definition of adversity. Only a level 3 study is available with fish. In the level 4 study with amphibians, no apical endpoints indicative of E and/or S were measured. The effects on amphibian gonads histopathology are considered indicative of E modality.	E, S (to be confirmed)
		Specific gonad histopathology in female (Ovary, increased granulomatous inflammation)		Effects observed at the highest tested concentration (0.0195 mg/L)	Effects observed at the highest tested concentration		
		Specific gonad histopathology in male		No effects observed up to the highest tested concentration	No effects observed up to the highest tested concentration		
	Sensitive to, but not diagnostic of, EATS	GSI (M and F)		Effects observed at the highest tested concentration (0.0195 mg/L)	Effects observed at the highest tested concentration		
		Reproduction (fecundity, fertility)		No effects observed up to the highest tested concentration	No effects observed up to the highest tested concentration		
LAGDA-OECD TG 241 *(Xenopus laevis)*	EATS mediated	Organ histopathology (Müllerian duct regression inhibited)	16	Effects observed in a dose response manner (0.02 mg/L)	Effects observed in a dose response manner		
		Organ histopathology (accelerated development of Müllerian duct)		Effects observed in a dose response manner (0.02 mg/L)	Effects observed in a dose response manner		
		Organ histopathology (minimal severity-changes in ovary histopathology)		Effects only observed at the highest tested dose (0.18 mg/L)	Effects only observed at the highest tested dose, in presence of systemic toxicity		
	Systemic toxicity	Liver weight		Evidence of systemic toxicity only at the highest tested concentration	Evidence of systemic toxicity only at the highest tested concentration		

Example 2

Test type (species)	Effect classification	Line(s) of evidence	Exposure (w)	Lowest Effect dose	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
AMA-OECD TG 231 (Xenopus laevis)	EATS-mediated	Developmental stage	1	0.017 mg/L	Effects were observed in a dose response manner	Overall the available evidence is considered to give positive evidence for endocrine activity through the T modality. The effects observed in the level 3 study (delay in development and decrease in hind limb length) were not confirmed in the level 4 study (LAGDA). Therefore, the available evidence is considered negative for adversity.	T
			3	0.067 mg/L			
	EATS-mediated	Hind limb length	1		No effects observed up to the highest tested concentration after 1 week. Effects only observed at the highest tested concentration after 3 weeks		
			3	0.341 mg/L			
	EATS-mediated	Thyroid histopathology (amphibian)	3	0.341 mg/L	Effects only observed at the highest tested concentration		
LAGDA-OECD TG 241 (Xenopus laevis)	EATS-mediated		16	0.18 mg/L			
AMA-OECD TG 231 (Xenopus laevis)	Sensitive to, but not diagnostic of, EATS	Body weight (amphibian)	1	0.341 mg/L	Effects only observed at the highest tested concentration		
			3	0.017 mg/L0	Effects were observed in a dose response manner		
LAGDA-OECD TG 241 (Xenopus laevis)			16		No effects observed up to the highest tested concentration		
AMA-OECD TG 231 (Xenopus laevis)	Sensitive to, but not diagnostic of, EATS	Snout-vent length/growth	1	0.341 mg/L	Effects only observed at the highest tested concentration		
			3	0.017 mg/L	Effects were observed in a dose response manner		
LAGDA-OECD TG 241 (Xenopus laevis)			16		No effects observed up to the highest tested concentration		
LAGDA-OECD TG 241 (Xenopus laevis)	EATS-mediated	Time to metamorphosis (NF stage 62)	16		No effects observed up to the highest tested concentration		
	Systemic toxicity	Liver weight		0.18 mg/L	Evidence of systemic toxicity		

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Lines of evidence for endocrine activity

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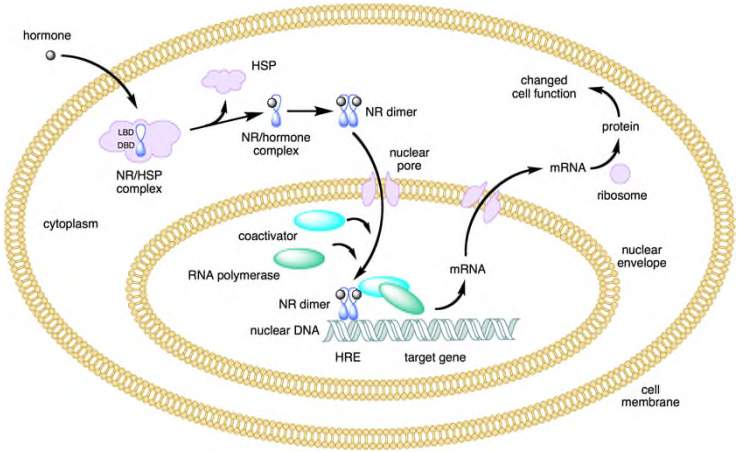
Testing for endocrine activity



Level 1/2

Level 3

MIE

Key Events

Adverse Outcome

Molecular Interaction → Cellular Response → Organ → Organ System → Organism → Population

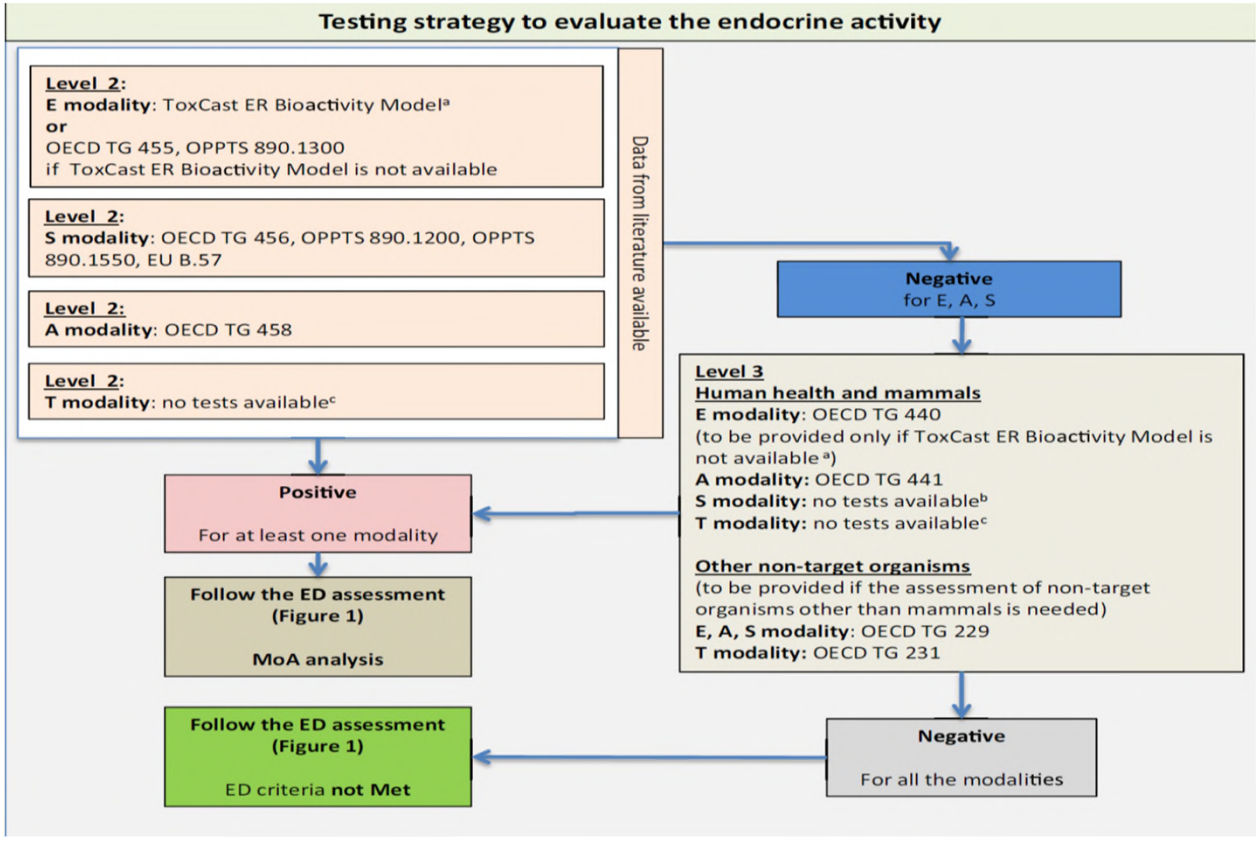
Bind to receptor → Receptor dimerization → Cofactor recruitment → DNA binding → Gene expression → Cellular response

OECD Conceptual Framework

Level 1	Existing data and non-test information
Level 2	In vitro assays providing data about selected endocrine mechanism(s) / pathways(s)
Level 3	In vivo assays providing data about selected endocrine mechanism(s) / pathway(s)
Level 4	In vivo assays providing data on adverse effects on endocrine relevant endpoints
Level 5	In vivo assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism

Testing strategy for endocrine activity

- Proposed tiered approach
  - Follow-up with level 2 tests
  - Follow –up with level 3 tests if negative level 2



Endpoints for endocrine activity non-target organisr

VTG

- Precursors of yolk proteins
- Produced by the liver under estrogenic regulation

Sex ratio

- Phenotypic and genetic sex

Spiggin

- Glycoprotein produced in the kidneys of sexually mature male three-spined sticklebacks
- Under androgen stimulation, normally not present in female

Secondary Sex Characteristics (SSCs) (where applicable)

- Male secondary sex characteristics (SSC) in females

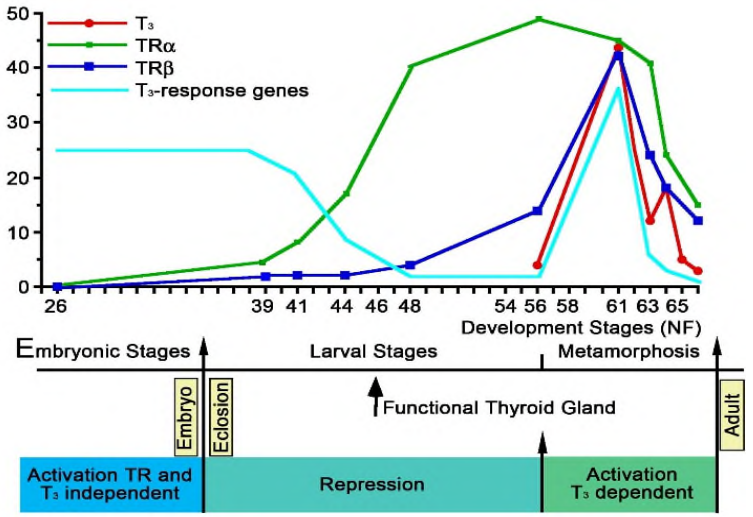
Level 3 tests – Fish

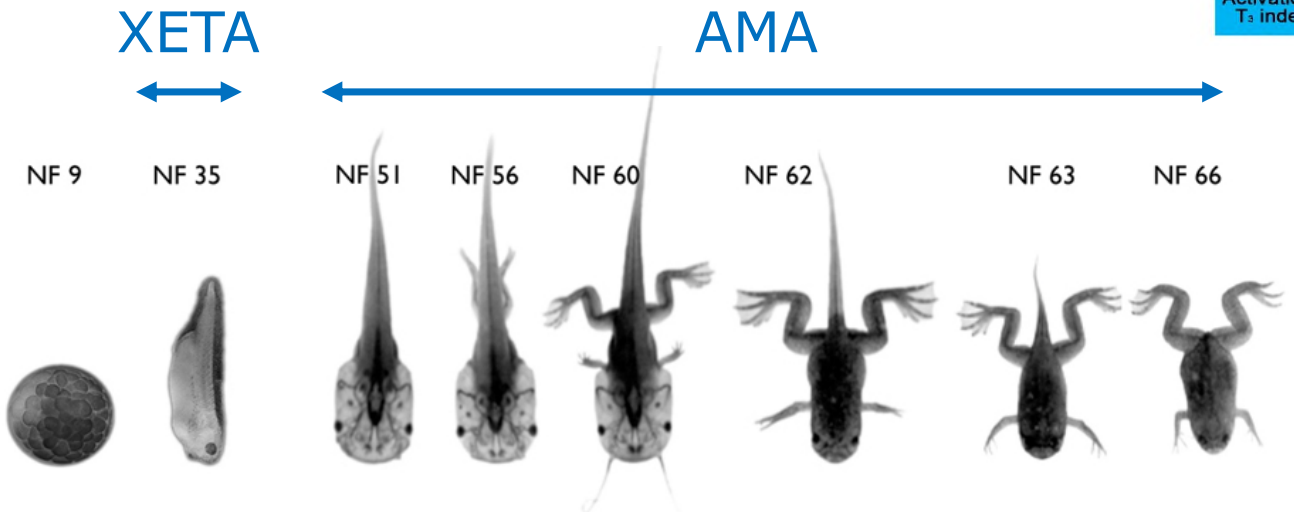
- 21-day fish assay (OECD TG 230)
  - Sexually mature male/spawning female, 21 days
  - VTG, SSC
- Fish short-term reproduction assay (OECD TG 229)
  - Sexually mature male/spawning female, 21 days
  - VTG, SSC, Specific gonad histopathology (fecundity)
- Androgenised female stickleback screen (OECD GD 148)
  - Female sexually mature sticklebacks, exposed to DHT
  - Reduction in (artificially induced) *Spiggin* production

All adult life stage!

Level 3 test - Amphibian (*Xenopus laevis*)

- XETA (OECD TG 248)
  - Eleutheroembryo, 72 hours
- AMA (OECD TG 231)
  - Larval stages, 21 days





Endocrine activity – example *in vivo*

	Grouping	Line(s) of evidence	Species/cell line(s)	Exposure (weeks)	Route of exposure	Effect dose (mg/L)	Observed effects (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
	In vivo mechanistic	Hormonal changes: oestradiol	*Pimephales promelas*	3	Water	0.5	Dose-dependent decrease	Sufficient: Oestradiol decrease observed in a dose-related manner but measured in one study only		S
		Vitellogenin (VTG) in females	*Pimephales promelas*	3	Water	1	Decrease only at the highest dose (large dose spacing; the previous dose is 0.12)	Sufficient: Dose-related changes in VTG. When the dose dependence could not be demonstrated, this is considered to be due to the test design (dose spacing and tested doses)		
			Pimephales promelas	3	Water	0.5	Dose-dependent decrease			
			Pimephales promelas	36	Water	0.558	Decrease only at the highest dose			

Lines of evidence for endocrine activity

- Is there evidence for endocrine activity?
  - Humans
  - Non-target organisms

- For which modality (or modalities)?
  - Group the effects per modality, and per effect (not e.g. per study)
  - Sort from upstream to downstream events

- Has endocrine activity been sufficiently investigated?
  - Add indicate when parameters have not been investigated

Conclusion on the assessment of endocrine activity

- Collect available data
  - Provide MoA information
  - Help with interpretation of *in vivo* data
  - Assays available for EAS, less for T

- Check available data
  - Dose-response of the data
  - Consistency among studies
  - Consideration of non-specific toxicity/effect, solubility, availability

WoE/expert judgement is required!

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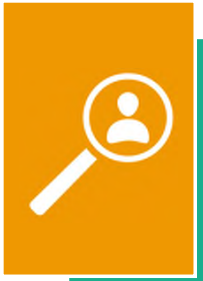

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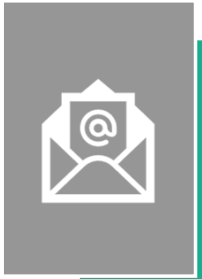

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