Trusted science for safe food

30 November 2020

Assembling and evaluating lines of evidence and definition of adversity

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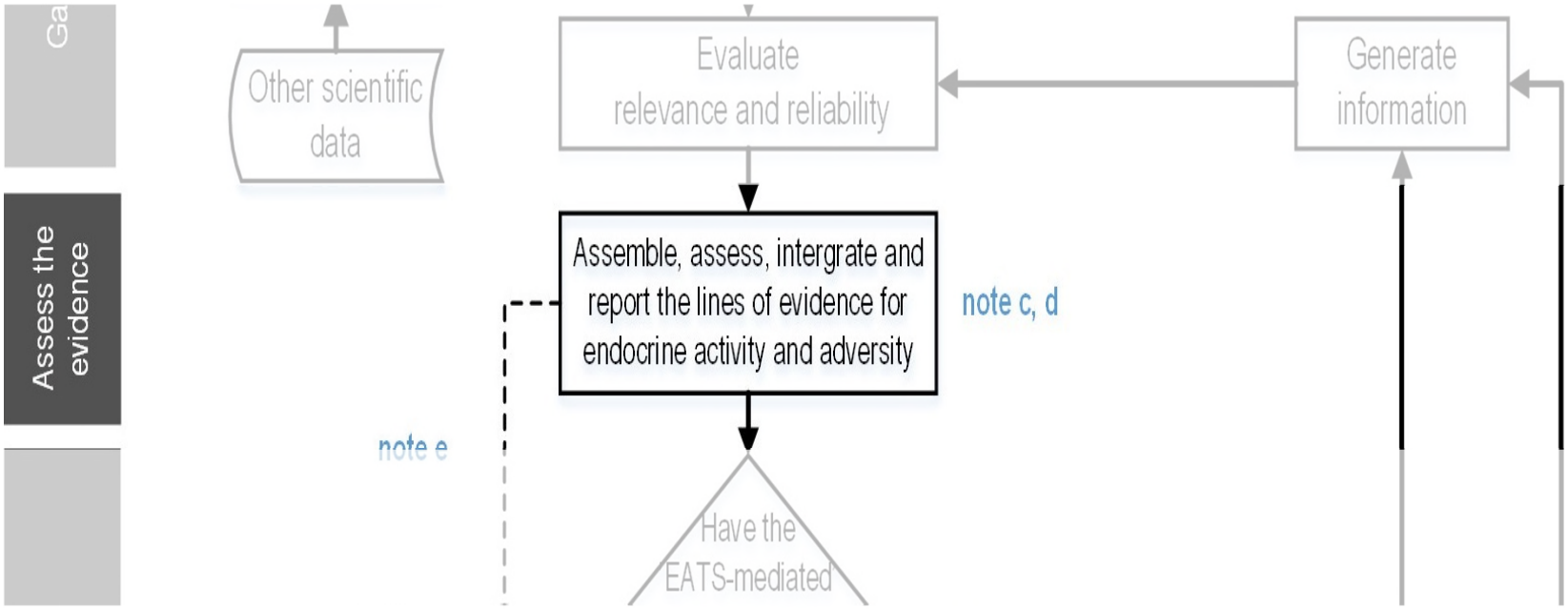
Assemble, Assess and Integrate the lines of evidence

II



Lines of evidence

- A line of evidence is a *'set of relevant information grouped to assess a hypothesis'*
- Respond to the problem formulation
  - Different subsets of information can be identified
- Need to consider positive and negative evidence
- Use information that is evaluated relevant and reliable
- **Parameters are grouped based on the potential to inform on EATS modalities according to the grouping (OECD GD 150)**:
  - EATS mediated'
  - 'sensitive to, but not diagnostic of, EATS'
  - In vitro mechanistic
  - In vivo mechanistic
- **Will be used to postulate MoAs**
- **Weight of evidence approach used**

Weight of evidence

The ED criteria state that a weight of evidence approach shall be applied for the assessment of the available scientific data

In the Guidance, weight of evidence methodology as indicated in the criteria is used in two different contexts:

- **Firstly, weight of evidence is applied for the evaluation of the line(s) of evidence for adversity and/or endocrine activity to determine whether there is sufficient empirical support for the assembled lines of evidence**; and
- Secondly, weight of evidence is used for the mode of action analysis, to establish the link between the adverse effect(s) and the endocrine activity.

Expert judgement could be necessary when considering the available lines of evidence, including the overall evaluation of the consistency of the dataset as a whole.

Lines of evidence for adversity

- Based on the WHO definition (WHO/IPCS, 2009)

  'A 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'

- Definition of adversity is generic (nothing specific to endocrine mediated effects)
- Current practices applicable to decide if observed effects are treatment related and adverse
- **Look for patterns of effects**
- Expert judgement may be needed

They will be integrated with the lines of evidence for endocrine activity.

Sources of information for evidence of adversity

- ✓Data generated using standardised test methods
- ✓Data generated using non-standard tests methods (provided it is of good quality)
  - Especially for non–EATS endocrine modalities
- ✓Systematic literature review
- ✓Epidemiological data
- ✓Data from other regulatory frameworks (e.g. REACH)
- ✓Read across and category approaches

Evidence for adversity

- **OECD CF Level 4 and 5** tests provide data on adverse effects on endocrine relevant endpoints **(see chapter 4 of the ED GD)**
  - **However, those parameters are not by default considered adverse and should be assessed according to a WoE approach**
- Certain parameters within **OECD CF Level 3** when measured in an intact animal model (e.g. Hershberger assay) may also provide additional information on adversity in certain circumstances

➢ **EATS-mediated parameters**: inform on adversity and are indicative of EATS MoA

➢ **Sensitive to, but not diagnostic of, EATS parameters**: inform on adversity but are not diagnostic on their own on EATS modalities

OECD CF Level 4

Level 4 **In vivo assays providing data on adverse effects on endocrine relevant endpoints**	• **Repeated dose 28-day study (OECD TG 407)** • **Repeated dose 90-day study (OECD TG 408)** • **Pubertal development and thyroid Function assay in peripubertal male rats (PP male Assay) (US EPA TG OPPTS 890.1500)** • **Pubertal development and thyroid function assay in peripubertal female Rats (PP female assay) (US EPA TG OPPTS 890.1450)** • **Prenatal developmental toxicity study (OECD TG 414)** • **Combined chronic toxicity and carcinogenicity studies (OECD TG 451-3)** • **Reproduction/developmental toxicity screening test (OECD TG 421)** • **Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422)** • **Developmental neurotoxicity study (OECD TG 426)** • **Repeated Dose Dermal Toxicity: 21/28-day Study (OECD TG 410)** • **Subchronic dermal toxicity: 90-day study (OECD TG 411)** • **28-Day (Subacute) Inhalation Toxicity Study (OECD TG 412)** • **Subchronic inhalation toxicity: 90-day study (OECD TG 413)** • **Repeated dose 90-day oral toxicity study in non-rodents (OECD TG 409)**

OECD CF Level 5

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**	• **Extended one-generation reproductive toxicity study (OECD TG 443)** • **2-Generation reproduction toxicity study (OECD TG 416 most recent update)**

Non-EATS modalities

- Adversity associated to sensitive to, but not diagnostic of, EATS parameters may be also a consequence of disruption in other endocrine modalities i.e. non –EATS
    - adversity in adrenals and/or pituitary can be caused by disruption in hypothalamic-pituitary –adrenal axis

- Standard tests may capture also other non-EATS related effects
    - Histopathological findings in the pancreas
    - Serum levels of corticosterone, insulin, glucose

- In isolation, EATS sensitive parameters are generally considered not sufficient for the definition of a pattern of adversity indicative of endocrine disruption with the assumption that EATS-mediated parameters would be more "sensitive and specific".

Assessment of adversity

Assessment is based on a WoE approach considering:

- Available data
  - Dose response
  - Temporal concordance
  - Consistency among studies and species
  - Repeatability
- Expert judgement

Lack of dose response or consistency should not lead to insufficient empirical support if it can be explained

- improper dose spacing
- differences in study design

Available epidemiological studies are only supportive evidence

- can never dismiss evidence for adversity from testing

Effects secondary to other toxicities

"adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor"

- The top dose of the toxicological study should:
  - be tolerated without inducing significant chronic physiological dysfunctions,
  - be compatible with animal survival
- Maximum tolerated dose (MTD) should be considered as a starting point for the evaluation of changes which could be due to excessive systemic toxicity
- MTD is a dose causing a minimum toxic effect considering alterations in physiological function, including:
  - no more than 10% decrease in body weight gain relative to control,
  - target organ toxicity and
  - alterations in clinical pathological parameters

- Expert judgement is necessary to define the MTD on a case-by-case basis

Effects secondary to other toxicities

These effects shall not be considered for the identification of the substance as endocrine disruptor

- When adverse effects only observed at excessive toxic dose (i.e. only observed above the MTD)
  - some specific considerations should be made when dealing with effects that are indeed also observable following endocrine imbalances
- When adverse effects observed at or below MTD if substantiated by MoA analysis

Human relevance

'A substance shall be considered as having endocrine-disrupting properties that may cause adverse effect in humans [. . .] unless there is evidence demonstrating that the adverse effects identified are not relevant to humans'

- the assessment of human relevance does not refer to adversity as such,

but rather

- to the question as to whether an effect elicited by a substance in a test animal could also be elicited in a human being

>> ***addressed in the MoA analysis***

Integration of Lines of Evidence

- Once assembled, the lines of evidence should be integrated for the assessment of adversity <u>for each modality</u>
- Information on systemic general toxicity or other target organs effects used to contextualise the presence of adversity
- The assessment of the integrated lines of evidence includes an evaluation of whether the data set is sufficient to support robust conclusion on adversity

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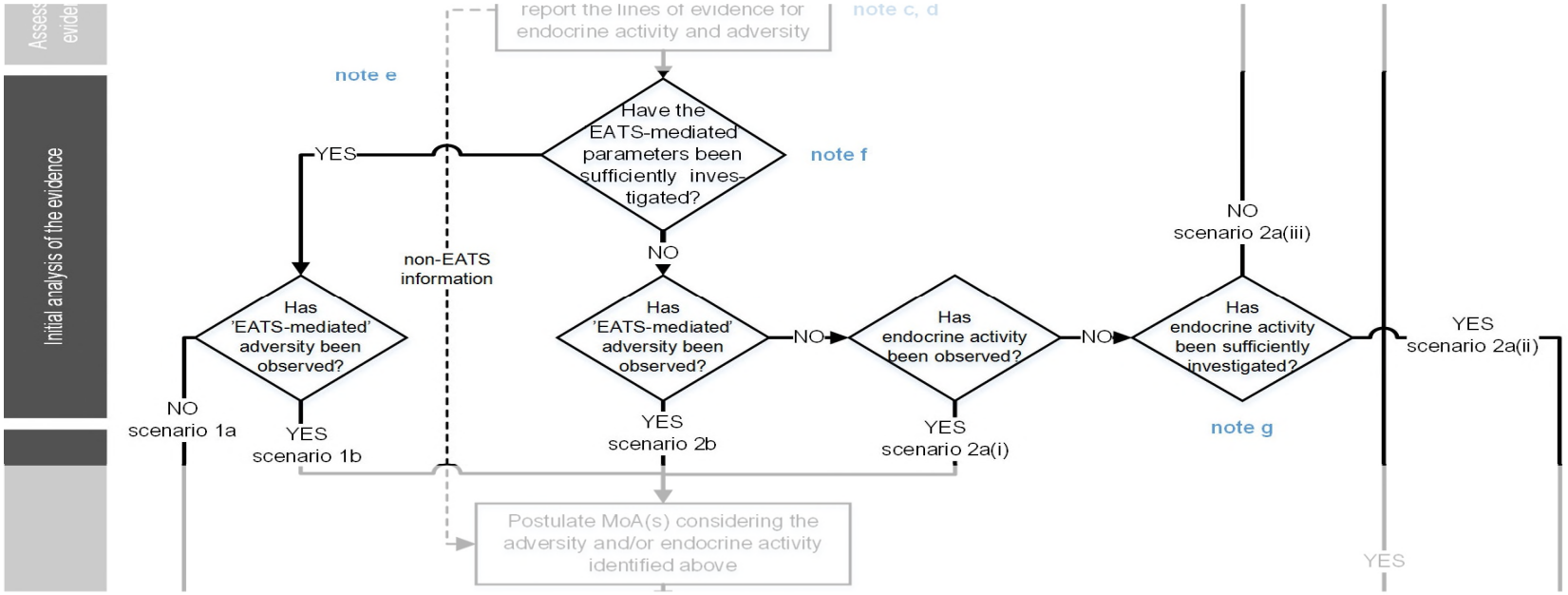

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Lines of evidence for endocrine activity

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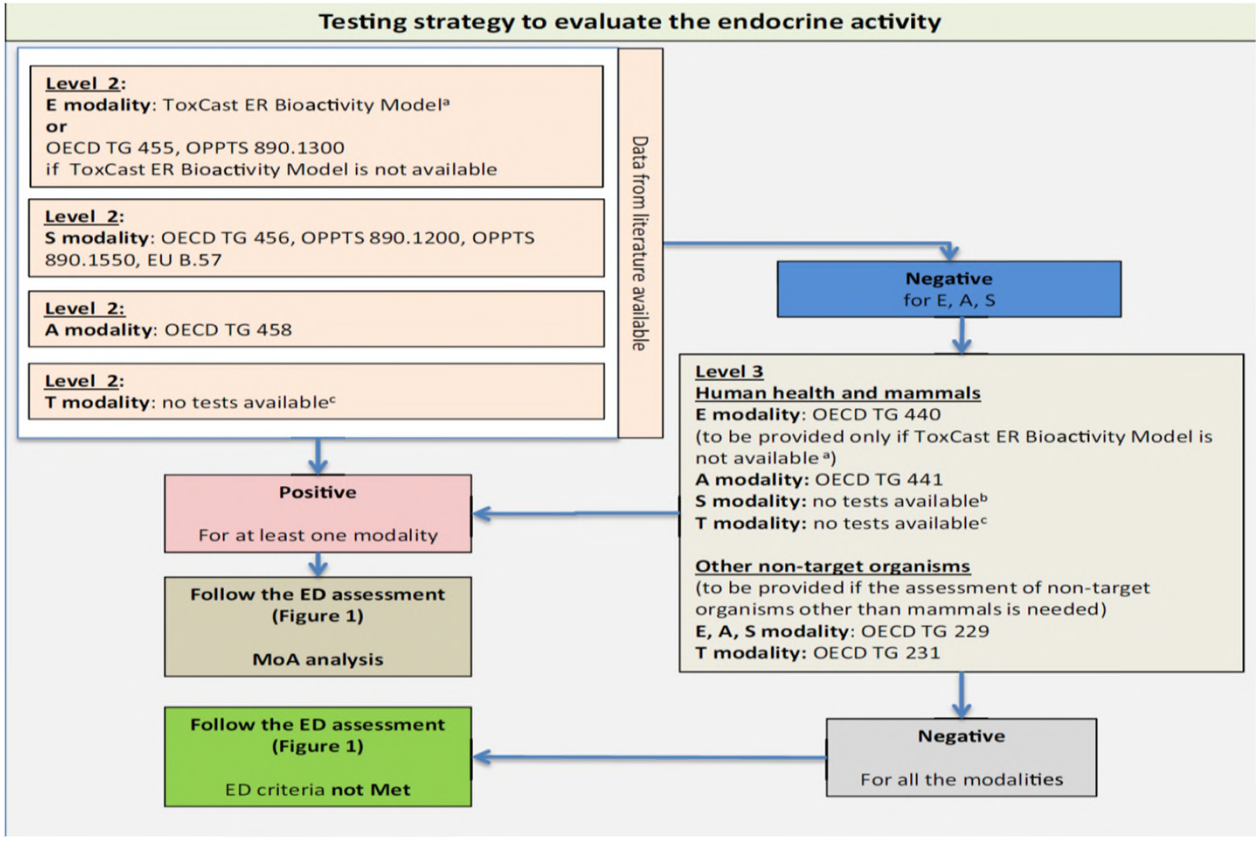
OECD Conceptual Framework

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



In silico models (level 1)

- (Q)SAR
- Molecular docking
- …

Appendix D - Databases, software tools and literature-derived (Q)SARs

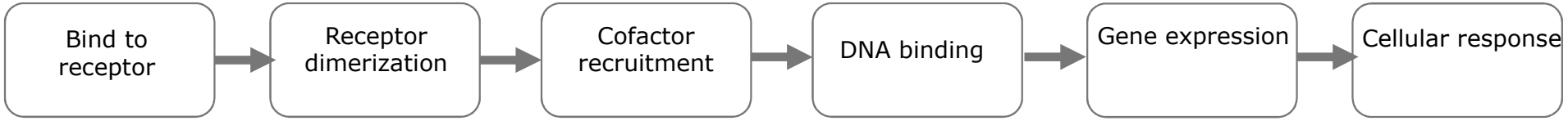
Add supportive information to in vitro mechanistic

Table 11: Software tools for predicting endocrine activity

Software tool	Effect addressed				
	E	A	T	S	Other
EDKB	X	X			
ADMET Predictor	X				
ACD/Labs Percepta – Toxicity Module	X				
Derek	X				
MolCode Toolbox	X				X[(a)]
CASE Ultra	X	X			
TIMES	X	X			X[(a)]
VirtualToxLab	X	X	X	X[(b)]	X[(c)]
OECD (Q)SAR Toolbox	X				
Endocrine Disruptome	X	X	X	X[(d)]	X[(e)]
COSMOS KNIME workflow	X	X	X	X[(d)]	X[(f)]
Danish (Q)SAR DB	X	X	X		X[(g)]
(Q)SAR Data Bank	X				
VEGA platform	X				

In vitro assays (level 2)

- **Receptor binding assay:** ligand-receptor interaction
- **Transactivation assay:** receptor activation leads to production of reporter gene product that can easily be quantified (e.g. luciferase, β-galactosidase)
- **Proliferation assay:** cell growth (proliferation) as a consequence of activity on a specific (endocrine) pathway



- **Enzyme inhibition assays:** inhibition of conversion or production of known compounds

Assays can be cell based or cell free (biochemical)

Endocrine activity – recommended tests

Table 4: Recommended tests methods to investigated EATS-related endocrine activity

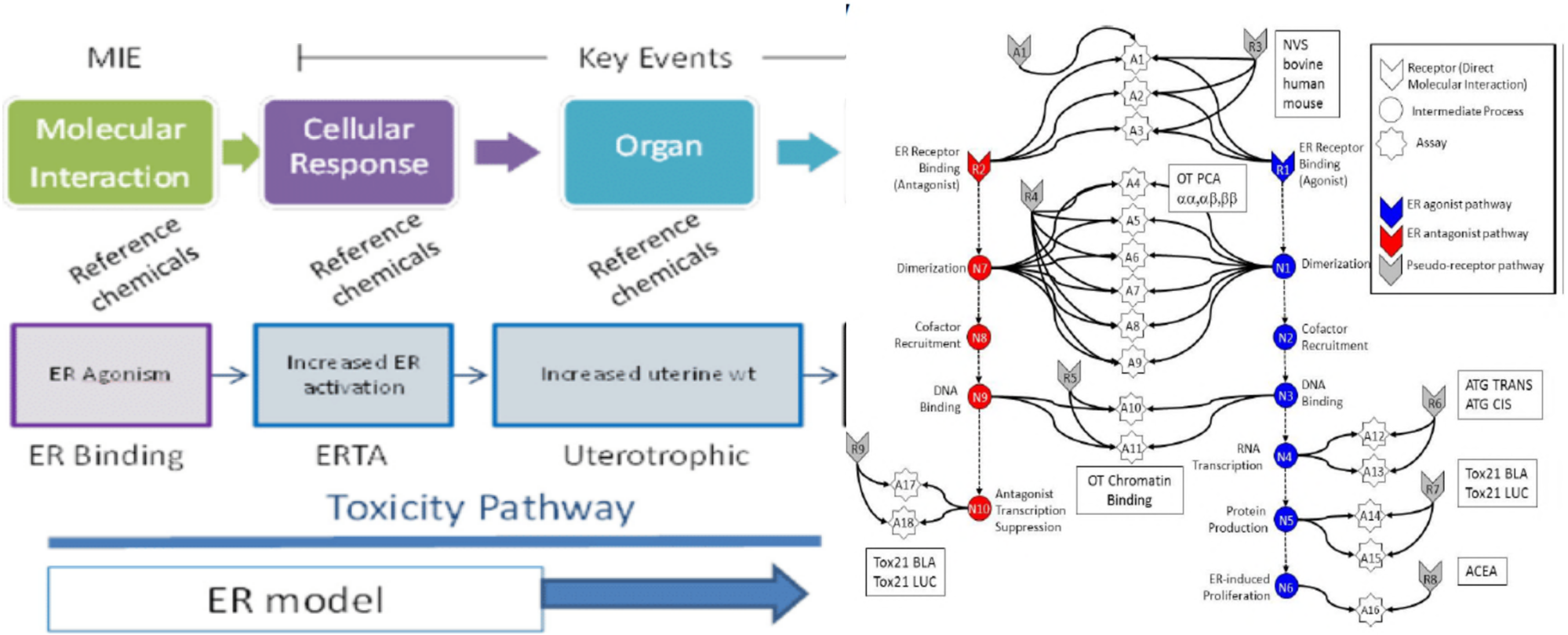
Pathway	OECD CF Level	Assay family	OECD guideline	Other guidelines	Other sources of data
Estrogen	Level 2	Transactivation assay	OECD TG 455	OPPTS 890.1300	ToxCast ER Bioactivity Model
	Level 3		OECD TG 440		
			OECD TG 229		
Androgen	Level 2	Transactivation assay	OECD TG 458		
	Level 3		OECD TG 441		
	Level 3		OECD TG 229		
Steroidogenesis	Level 2	Steroidogenesis	OECD TG 456	OPPTS 890.1550; EU B.57	
	Level 3	CYP19	OECD TG 229	OPPTS 890.1200	
Thyroid	Level 3		OECD TG 231		

No validated (OECD TG) assays available

Special case of level 2: ToxCast

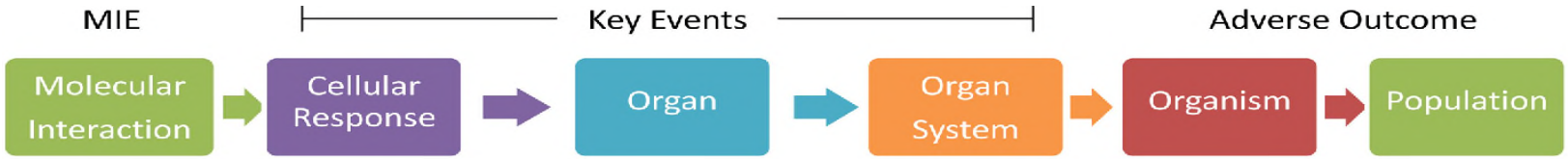
- TOXicity ForeCASTer
  - Prioritize, screen and evaluate thousands of chemicals
  - Includes data on many biocides (and pesticides)
  - Includes many in vitro assays relevant for ED
  - Data publically available (incl. QC) but not (all) published in scientific literature
- Generate predictive models based on this data
  - ER model, AR model
- ToxCast subset EDSP21 is not the same as assessed under EDSP
  - EDSP: 5 specific in vitro assays + level 3 assays
  - EDSP21: subset of ED relevant ToxCast assays

ToxCast ER model



- Predict *in vivo* (level 3) on the basis of *in vitro* (level 2)
- Available for estrogen action (and androgen action)
  - Predict Uterotrophic assay (Hershberger assay)

In vivo mechanistic (level 3)



- Specific effects (e.g. organ weight)
  - Considered indicative of ED effects (yes/no answers)
  - Not (necessarily) adverse (but could provide indications)
- Hormone levels (OECD level 3, 4 and 5):
  - Estradiol, Testosterone, T3/T4, fT4, FSH, LH, TSH
- Relatively short term
- Animals made more sensitive (e.g. non-intact) and/or specific life stage

Level 3 tests – mammalian tests

Uterotrophic assay

- Estrogenic (OECD TG 440) and anti-estrogenic (OECD GD 71) effects
- Immature or ovariectomised young adult female rats
- Endpoints: uterine weight and histopathological changes in the uterus and vagina

Hershberger assay

- Androgenic and anti-androgenic effects
- Immature (OECD GD 115) or castrated peripubertal (OECD TG 441) male rats
- Endpoints: weight of ventral prostate, seminal vesicles, LABC, Cowper's glands and glans penis

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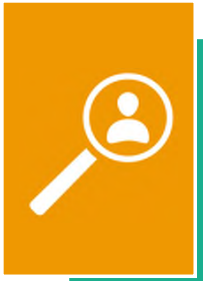

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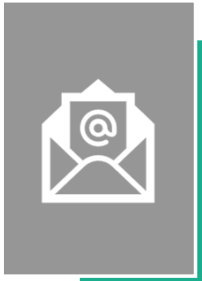

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