OECD Activities on Endocrine Testing

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OCDE
DES POLITIQUES MEILLEURES POUR UNE VIE MEILLEURE

OECD Test Guidelines for detection of EDCs

Test Guidelines Number and Name*	Level of Conceptual Framework	Pathway Addressed			
		Oestrogen	Androgen	Thyroid	Steroidogenesis
TG 493: *In Vitro* Oestrogen Receptor Binding Assay	2	X			
TG 455: *In Vitro* Oestrogen Receptor Transactivation Assay	2	X			
TG 458: *In Vitro* Androgen Receptor Transactivation Assay	2		X		
TG 456: H295R Steroidogenesis Assay	2	X	X		X
TG 440: Uterotrophic Bioassay	3	X			
TG 441: Hershberger Bioassay	3		X		
TG 229: Fish Short-Term Reproduction Test	3	X	X		X
TG 230: Fish Screening Assay	3	X	X		X
TG 231: Amphibian Metamorphosis Assay	3			X	
TG 407: 28-day Repeated Dose Toxicity Study in Rodents	4			X	X
TG 408: 90-day Repeated Dose Toxicity Study in Rodents	4			X	X
TG 414: Prenatal Developmental Toxicity Study	4	X	X	X	X
TG 421: Reproduction/Developmental Toxicity Screening Test	4	X	X	X	X
TG 422: Combined Repeated Dose Reproduction/Developmental Toxicity Screening Test	4	X	X	X	X
TG 426: Developmental Neurotoxicity Study	4	X	X	X	X
TG 451-3: Combined Chronic Toxicity/Carcinogenicity Study	4	X	X	X	X
TG 234: Fish Sexual Development Test	4	X	X		X
TG 241: Larval Amphibian Growth and Development Assay	4			X	
TG 443: Extended One-Generation Reproductive Toxicity Study	5	X	X	X	X
TG 240: Medaka Extended One-Generation Reproductive Toxicity Study	5	X	X	X	X
TG 416: Two Generation Reproduction Toxicity Study	5	X	X	X	X

*Test guidelines may evolve following best practices and emerging science.

OECD Projects for EDCs

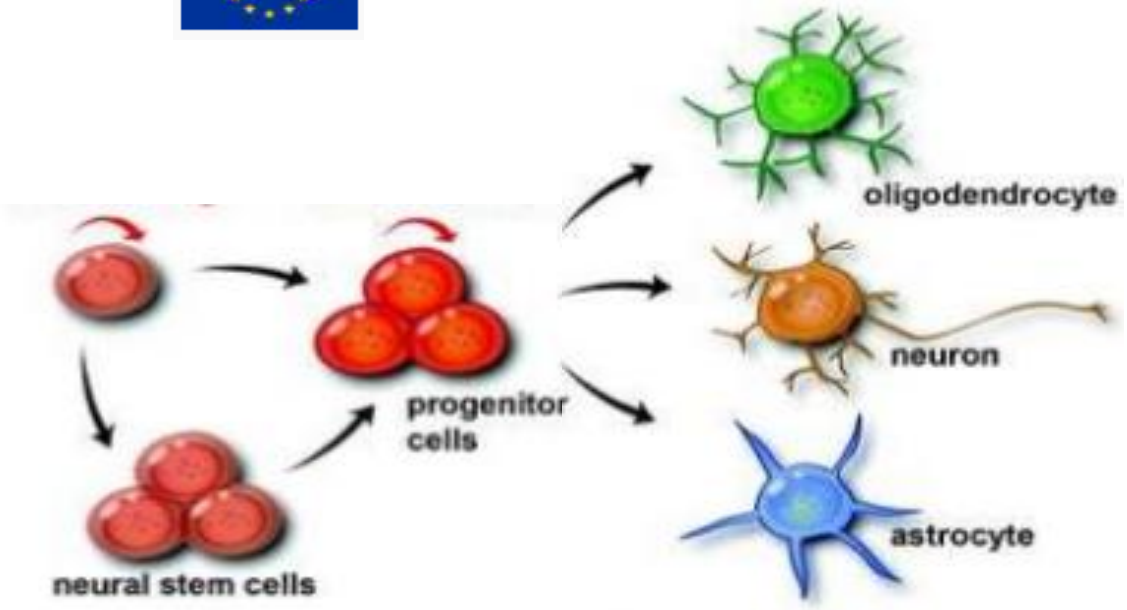
- New/Updated TGs
  - TG 458: In vitro Androgen Receptor Transactivation (2020)
  - TG 248: Xenopus Eleutheroembryonic Thyroid Assay (2019)
- Ongoing projects
  - New TG for the Detection of Endocrine Active Substances acting through ER using transgenic cyp 19a1b-GFP Zebrafish Embryos (EASZY assay) (FR)
  - Zebrafish Extended One Generation Reproduction Test (ZEOGRT) (GER)
  - New TG RADAR assay – Rapid Androgen Disruption Animal Replacement assay (FR/UK)
  - Inclusion of thyroid endpoints in OECD fish tests (DK)
  - REACTIV (Rapid Estrogen Activity In Vitro) Assay (FR)
  - Guidance Document on Juvenile Medaka anti-androgen screening assay (JP)
  - Detailed Review Paper on Embryonic Stem Cell assays (JP)
  - Guidance Document on Developmental Neurotoxicity (DNT) in vitro assays (EU/US/DK)
  - Detailed Review Paper on the State of the Art of Metabolic Disruption by Chemicals (NL/UK/SWE/GER/DK)
  - Detailed Review Paper on Retinoid signaling pathway (SWE/Secr.)

Retinoid signalling pathway

Retinoid signalling critical for development

- Regulation cell proliferation and differentiation
- Effects on neural tube development
- Axial patterning
- Germ cell differentiation/development

Funding from DG ENV



- OECD project to develop a Detailed Review Paper focusing on retinoid signalling effects on
  - Male reproduction
  - Female reproduction
  - CNS
  - Skeletal/craniofacial development
- **Objectives:**
  - Review retinoid pathway signalling biology
  - Identify possible new in vitro/in vivo assays + new endpoints for existing in vivo assays
- Status
  - September 2019– draft circulated for comments
  - November 2019–meeting to make recommendations on
    - Assays for development-Regulatory context
    - next steps
  - April 2020 – revised draft to EDTA
  - September 2020 – draft to retinoid EG
  - December 2020 – revised draft expected for comment

Development of new or modified Test Guidelines

1. Proposal submitted by a MC or representative of EC
   - Reviewed by the Working Group of National Coordinators for the Test Guidelines Programme (WNT)
   - Must have a clear regulatory application
2. Technical work may taken up/reviewed by an OECD Expert Group
   - EDTA, VMG-Eco, VMG-NA
3. The method goes through a rigorous and robust process before being accepted
   - Timeline can be ~18 mo to 5+years
4. Guideline must be “fit for purpose”

Time Required To Validate an Assay/Approve a Test Guideline

- Varies depending on:

Factors that can slow down the process

  - Preparatory work on the standardisation of the assay and testing procedures
  - Identification of reference chemicals
  - Availability and distribution of test chemicals/test system components
  - Recruitment of participating laboratories

  - Sense of urgency on the regulatory need
  - Availability of preliminary data/detailed description of test method/reliability/relevance/link to regulatory endpoint/ preliminary data

Factor that can speed up the process

How to facilitate researchers/method developers/uptake of methods

Understanding regulatory context/international regulatory requirements

Follow best practices

- Good In Vitro Methods Practices (No 286)
- Guiding Principles on Good Practices for the Availability/Distribution of Protected Elements in OECD Test Guidelines (No 298)
- Guidance Document for Describing Non-Guideline In Vitro Test Methods (No 211)
  - OECD Harmonised Template for intermediate effects (OHT 201)

Data

- Validation data
- Transparency of reference chemical selection
- Clear link to regulatory endpoint

Evolution of OECD Guidelines

Test Guideline

- Include a single test method
- Validated using relatively few model (reference) chemicals

PBTG

- Include >1 method that are technologically and functionally similar
- **Measure the same target and technology**
- Validated using Performance Standards

KETG

- Include >1 method that are not technologically similar
- **Measure the same key event**

DA GL

- Include >1 method that are technologically and functionally diverse
- Methods used in combination to **predict the same adverse effect**
- Can be in silico/in vitro/in chemico combined with a fixed DIP

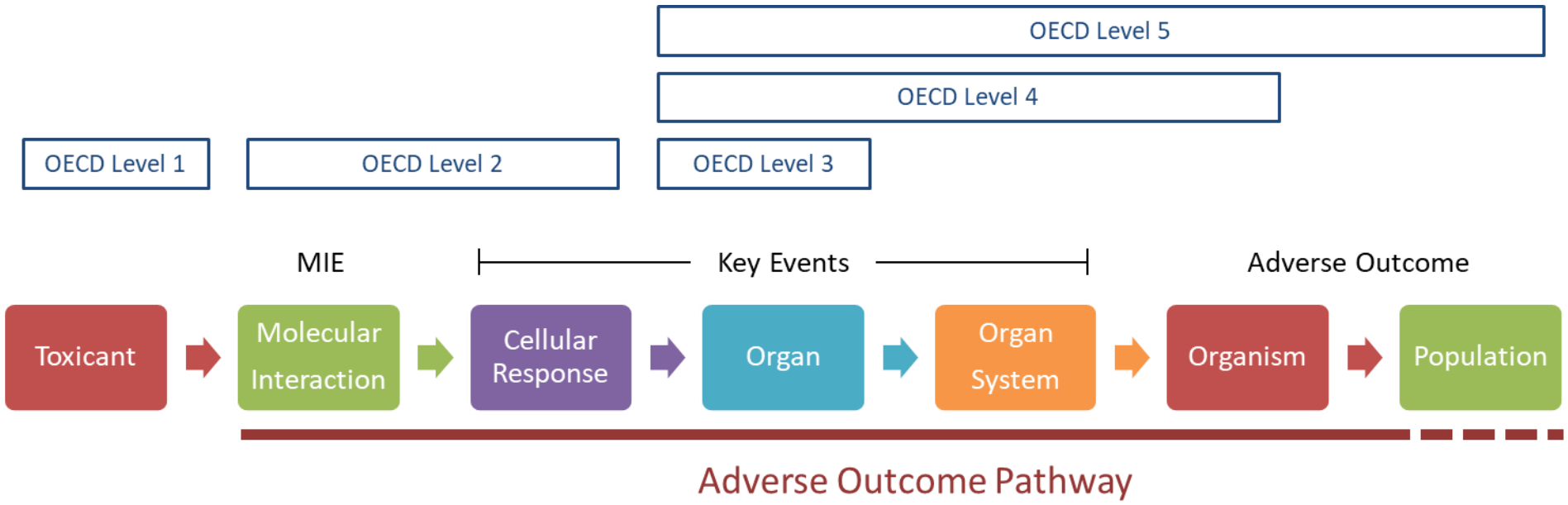
Innovative test methods

- Increasingly number of in vitro and in silico methods
  - can be **integrated** to develop **new approaches** for evaluating chemical safety
  - reflect an **increasing understanding in the underlying biology**
- Integrate results from multiple methods
  - May include different points in a pathway and may use different technologies
  - Overcome the limitations of individual assays

New Approaches to Endocrine Testing

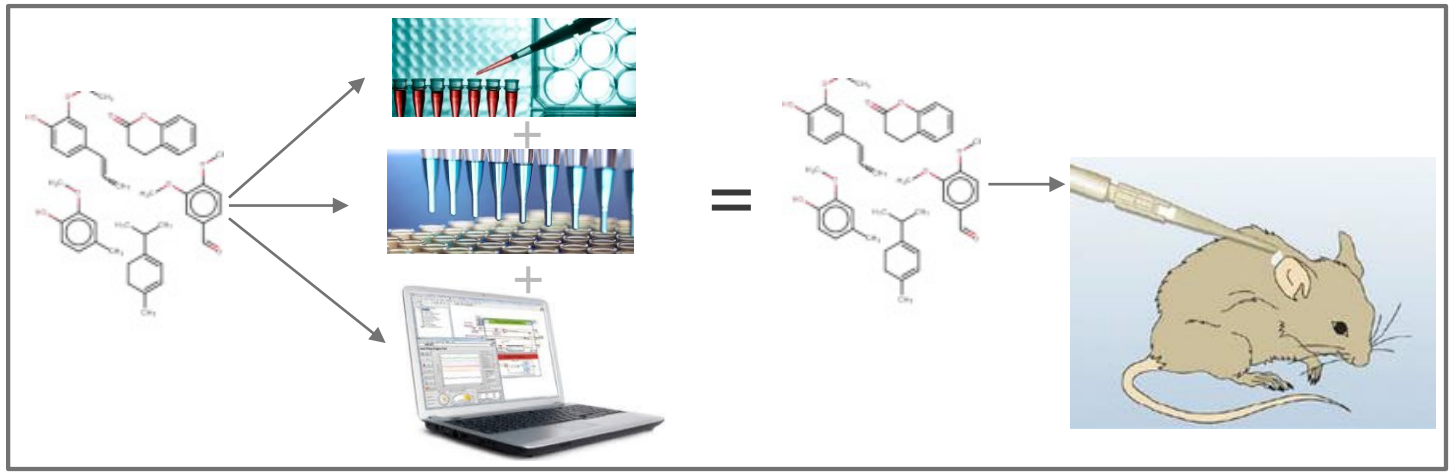
- To date, no single assay provides information on a chemical's:
  - toxicity mode of action (i.e. mechanistic information)
  - apical response used in regulatory decision making
- Regulatory programmes use multiple methods in combination to evaluate potential endocrine activity
  - Requires evidence integration
    - Natural fit for IATA/AOPs

Endocrine Disruptor Screening and Testing Assays



Alternative methods used in combination

- Defined Approaches leverage the strengths of individual methods (e.g. some are better at some jobs than others)



Individual methods A + B + C

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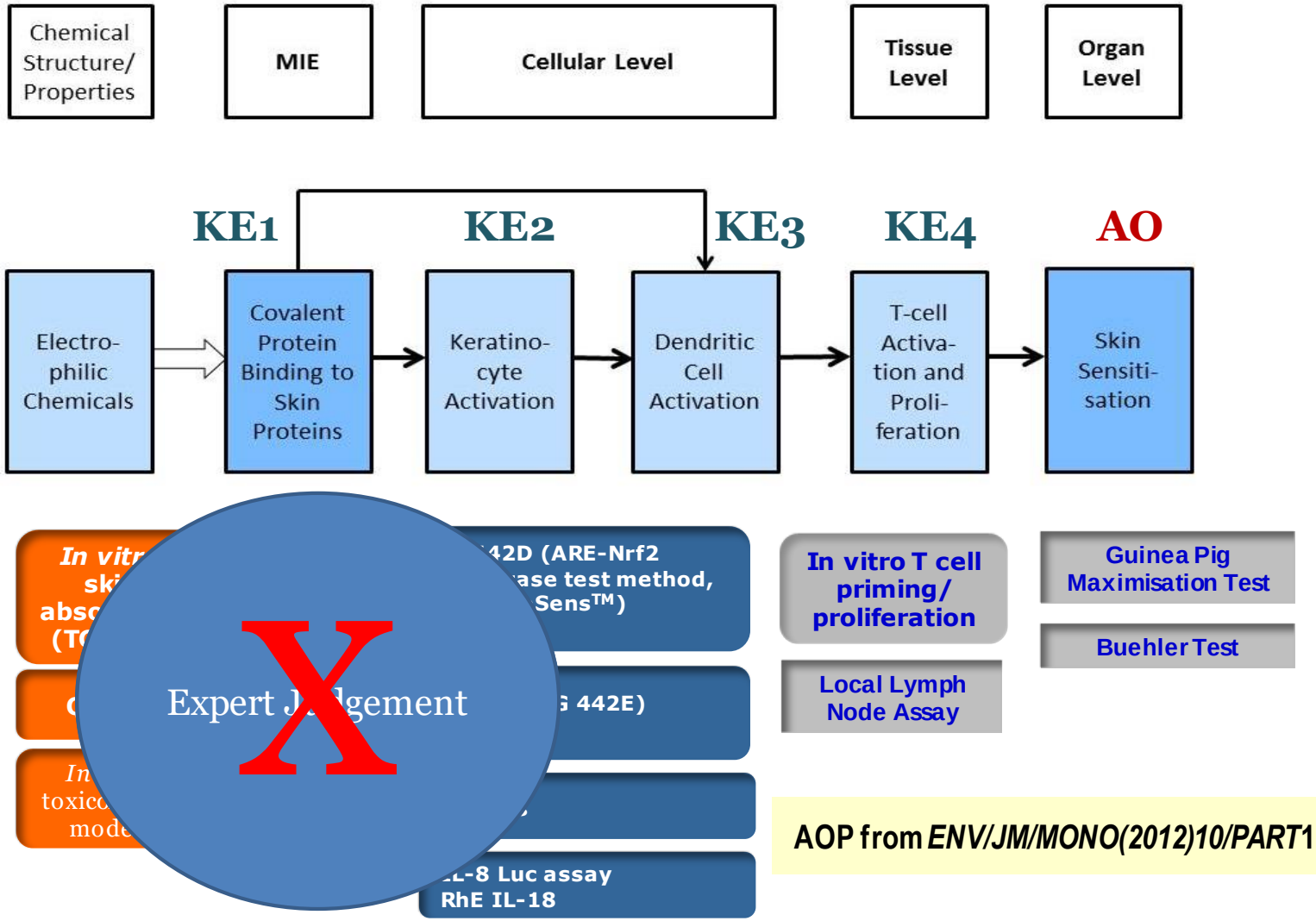
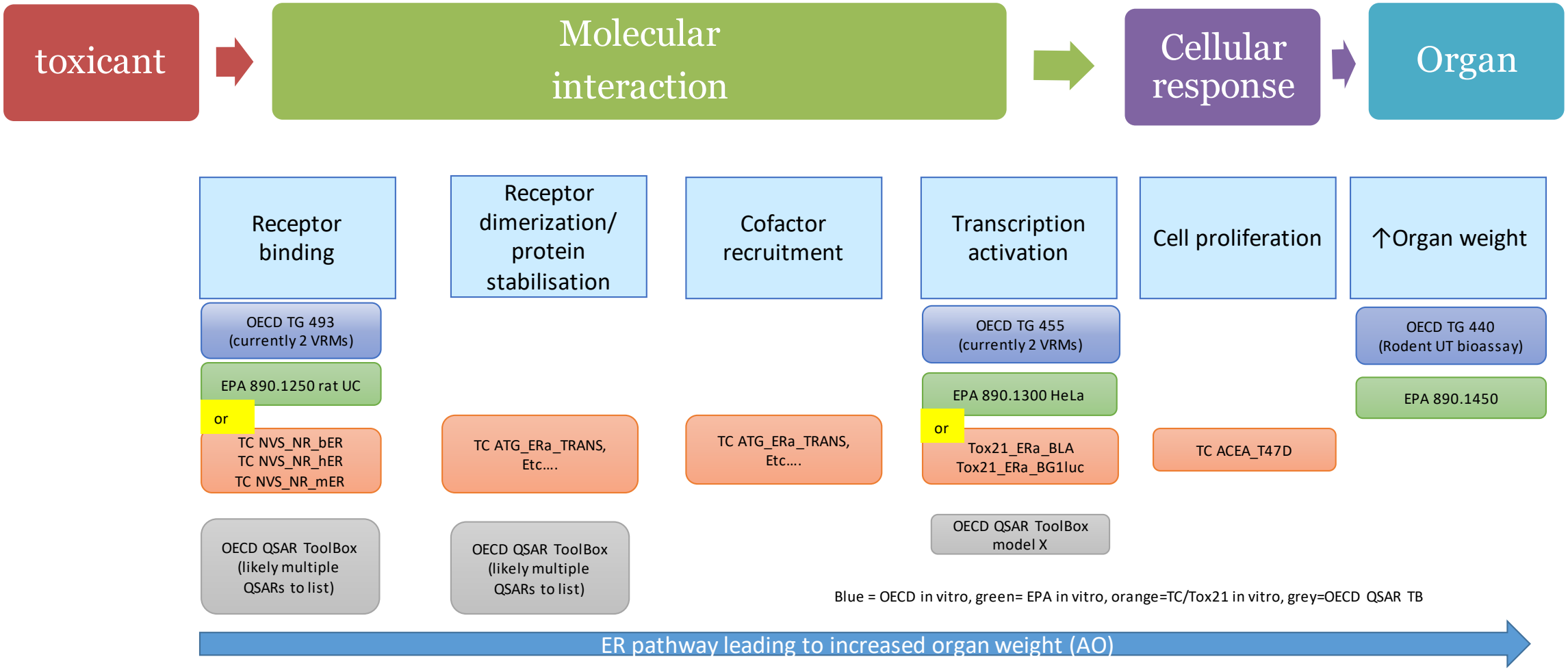
Predicts the in vivo reference method!

Follow example from Skin sensitization: complete AOP + alternative method toolbox



https://aopwiki.org/wiki/index.php/Aop:40

Testing strategy developed around an AOP: ER pathway



ER IATA case study predicting rodent Uterotropic response published 2019

IATA versus Defined Approaches

IATA	Defined Approaches
Designed in response to problem formulation	Designed to address pre-defined endpoint/prediction
Inputs are defined by user	Defined information sources
Sequence of input, next steps, decision context defined by user	Sequence defined and next steps are rule-based
Expert judgement for weighting data, interpreting data	Fixed data interpretation procedure
Conclusion may be open to interpretation	Regulatory conclusion is clear

Efforts to address a current challenge

- Different terms are used to describe the same biology
  - OECD Test Guideline endpoints
  - AOP ontology
  - OECD QSAR ToolBox ontology
  - Beginning discussion on search strategies/systematic literature review (in the context of endocrine disruption)

Summary

Test Guideline	Process	Object	Action
• TG 455 • TG 458 • TG 493 • TG 456	• Receptor-mediated gene expression • Receptor-mediated gene expression • Nuclear receptor binding • Steroid metabolism	• Estrogen receptor alpha • Androgen receptor • Estrogen receptor alpha* • Steroidogenic enzymes	• Increased/decreased/NE (equivocal?) • Increased/decreased/NE (equivocal?) • Binder/non-binder= NE (equivocal) • Inducer/inhibitor/NE (equivocal?)

The variable terminology was generally descriptors for **action** (or both **process** and/or **action**, depending on the context);
e.g. for TG 458, the following terms were used to describe actions (or process/action)

increased	**decreased**	**either**
Agonism	Antagonism	Anti(androgens)
Agonist	Antagonist	(Anti)androgenic activity
Agonistic	Antagonistic	(Anti)-androgenic activity
Androgen activity	Anti-androgen activities	Transactiavtion assay
Androgenic activity	Antiandrogenic	Transcriptional activity
Induction (of activity)	Antiandrogens	Transcriptional activation
Induced	AR blocking	Transcriptional response
	Inhibition (of activity)	AR-mediated transactivation/inhibition
	Inhibitory responses	Signal activation/blocking
	Decrease in activity	

Ontologies:
Better interoperability through controlled vocabulary

- To find common ontology for
  - OECD GL endpoints
  - MIEs/KEs in AOP
  - Targets in OECD QSAR ToolBox
  - Search terms for literature review
  - OHTs
- Outcomes
  - Connection between OECD GL and AOPs
  - Data associated with MIE/KE
  - If endpoints used for regulatory assessment, A LOT of data
- Potential for
  - Identifying reference chemicals for new method validation
  - building better predictive models
  - IATAs and DAs to predict apical/in vivo endpoints

Other types of OECD projects and ways to engage

- Guidance Documents or Detailed Review Papers
  - Also part of the Test Guidelines Programme
- AOPs
  - Project submission form; coordinated with WNT
  - Reviewed by EAGMST and if supported, added to the AOP work plan
  - Reviews in June and December
- IATA Case Studies
  - Projects proposed in Nov
  - Case studies submitted in March
  - Reviewed by IATA group and discussed in June
    - Authors give a short webinar to aid reviewers
  - Case study revised/finalised

Thank you! Questions?

OECD Work On
Endocrine Disrupting Chemicals

BETTER POLICIES FOR BETTER LIVES