In vitro assays for endocrine disruptors: test performance and steps to validation.

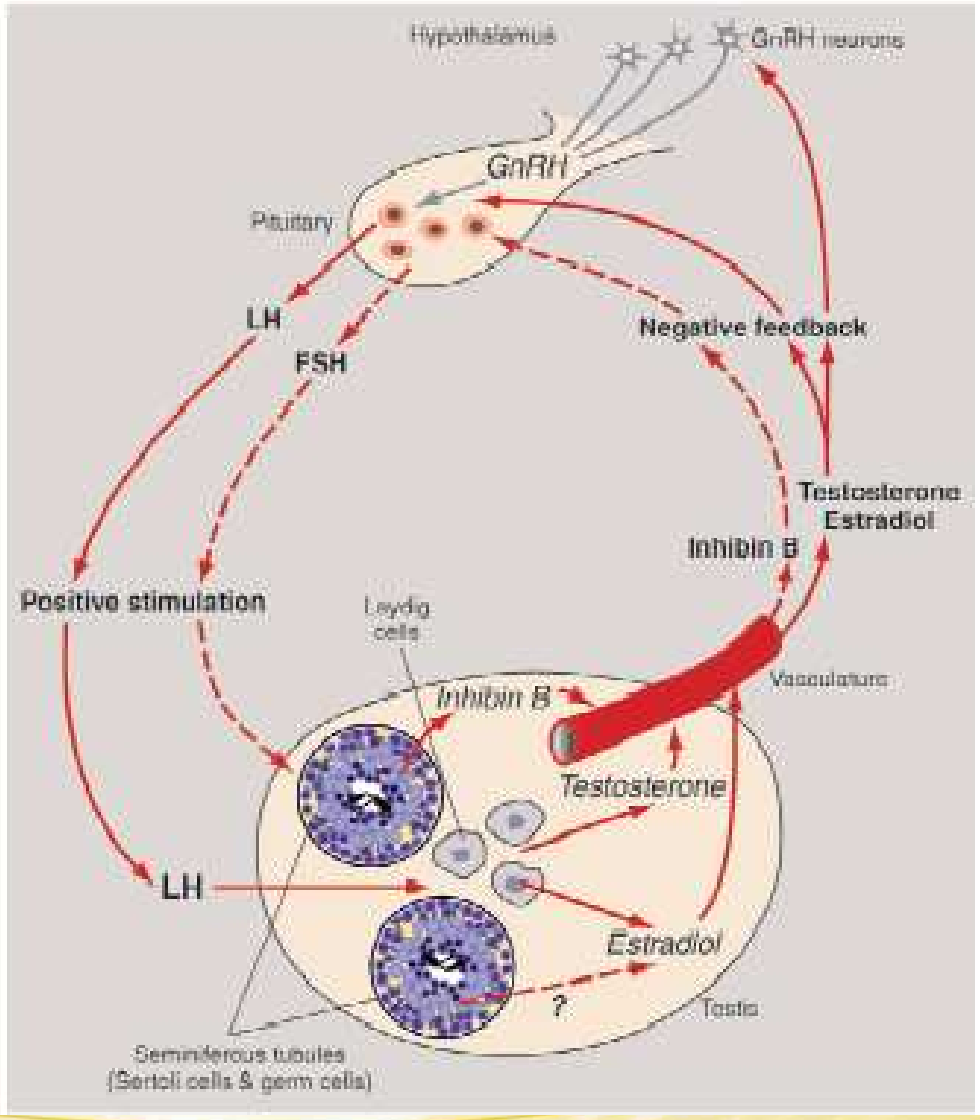
Witters Hilda

Overview

- Background ED
- Validation of *in vitro* tests
- ER transactivation assay
- Conclusions and next steps

- **Man-made chemicals & natural compounds**
  - Interfere with endocrine system
  - Adverse effects on wildlife and humans

The endocrine system



Source: WHO/IPCS,2002

- Man-made chemicals & natural compounds

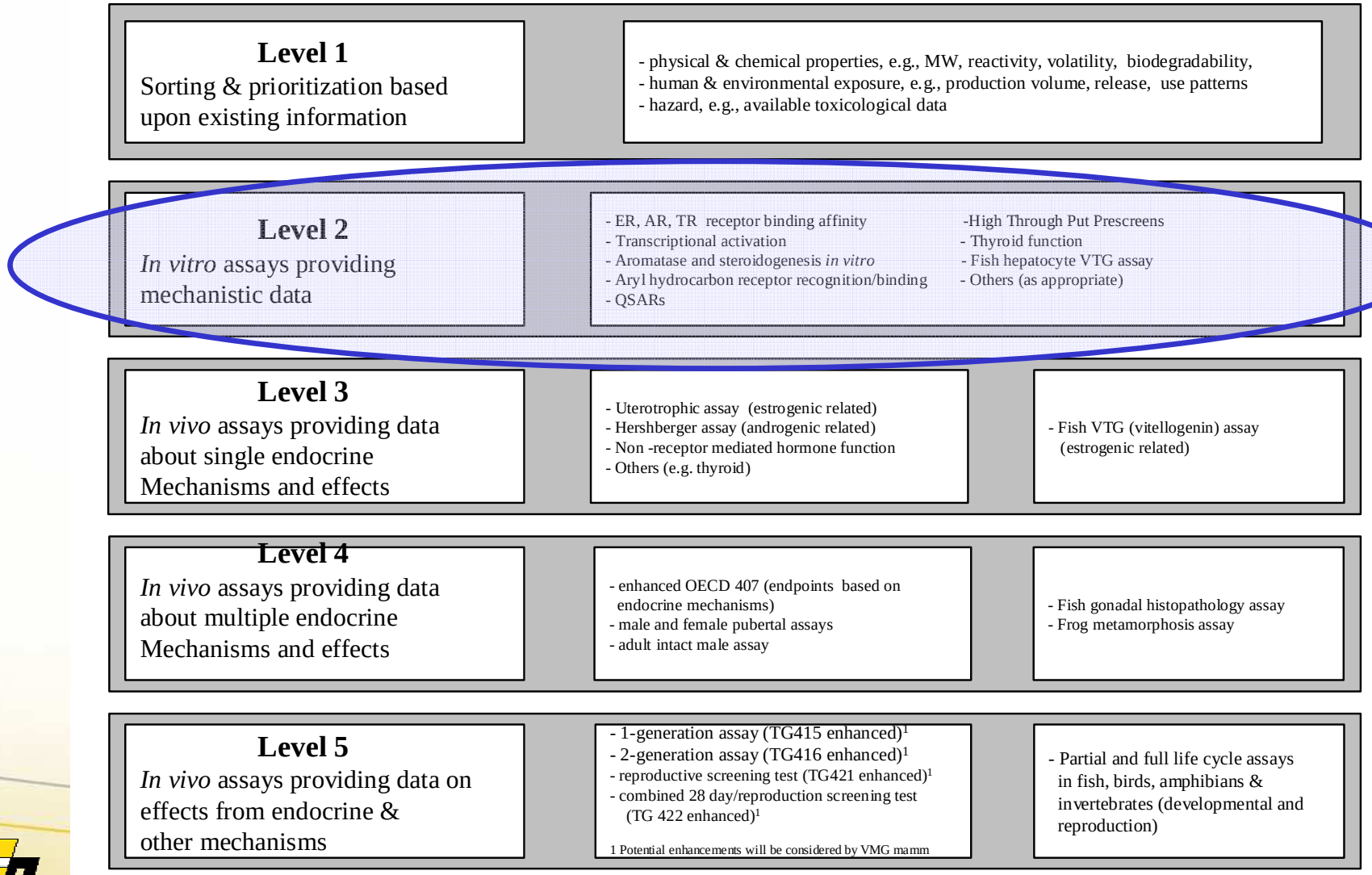
- **Testing needs**
  - US EPA → EDSP (1998)
  - OECD → conceptual framework for testing (2002)

  → tiered testing programme

Background

Note: Document prepared by the Secretariat of the Test Guidelines Programme based on the agreement reached at the 6th Meeting of the EDTA Task Force

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals



- Man-made chemicals & natural compounds
- Testing needs
- ***In vitro* mechanistic studies**
  - Extended panel of tests, reviewed (ICCVAM; OECD)
  - Receptor based tests→ recommended for validation

Study design: ED

Management team
B. Stokes (ICCVAM/US)
P. Pazos/M. Jacobs (ECVAM/EU)
A. Mantovani (ISS/IT)
C. McArdle (Univ. of Bristol, UK)

Chemical Selection
K. Aschberger (ECB)

Protein binding assays

ER binding assay (Pan Vera)
1. lab: Research Triangle Park/US EPA)
2. lab: Bayer/ Germany
3. lab: CERI/Japan

AR binding assay (Pan Vera)
1. lab: Bayer/ Germany
2. lab: Research Triangle Park/US EPA
3. lab: Institute of Applied Microbiology /A

Transcriptional activation assays

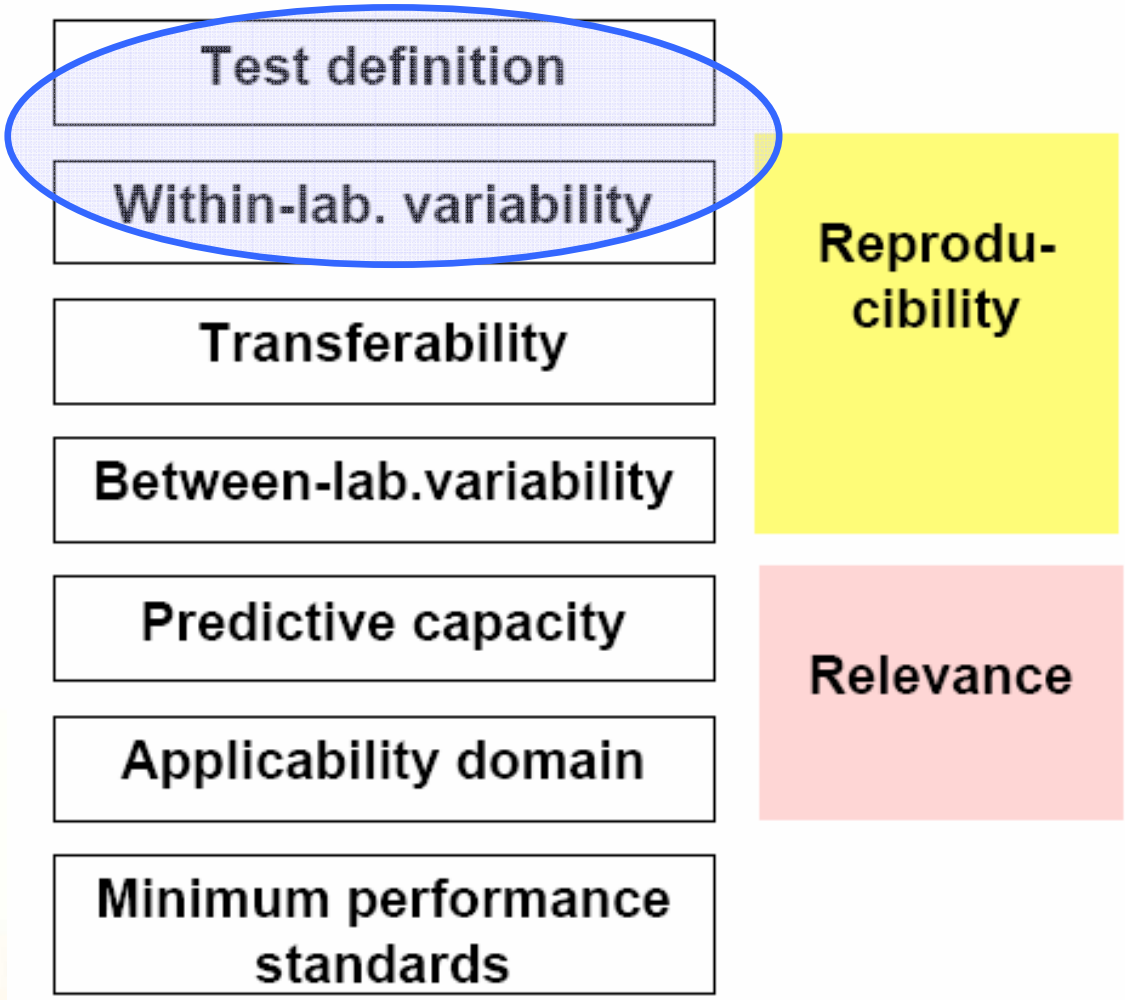
MELN (MCF-7 cells)
1. lab: Vito/B
2. lab: Bayer/D
3. lab: NN

PALM (PC-3 cells)
1. lab: Bayer/D
2. lab: Vito/B
3. lab: NN

ER-Calux (USO2 cells)
1. lab: Bio Detection System/NL
2. lab: Vito/ B
3. lab: Bayer

AR-Calux (USO2 cells)
1. lab:Bio Detection System/NL
2. lab: Vito/ B
3. lab: Bayer/D

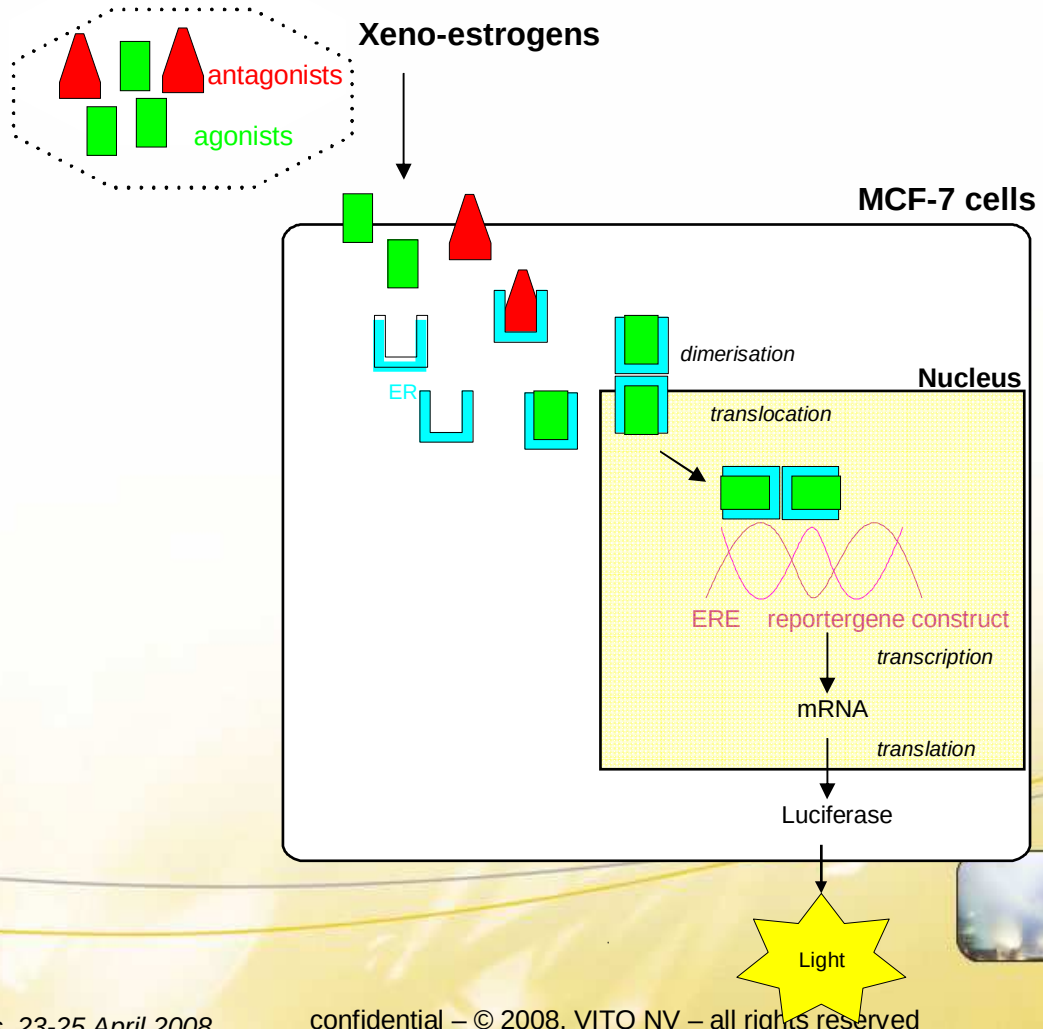
Validation



Source: Hartung et al., 2004

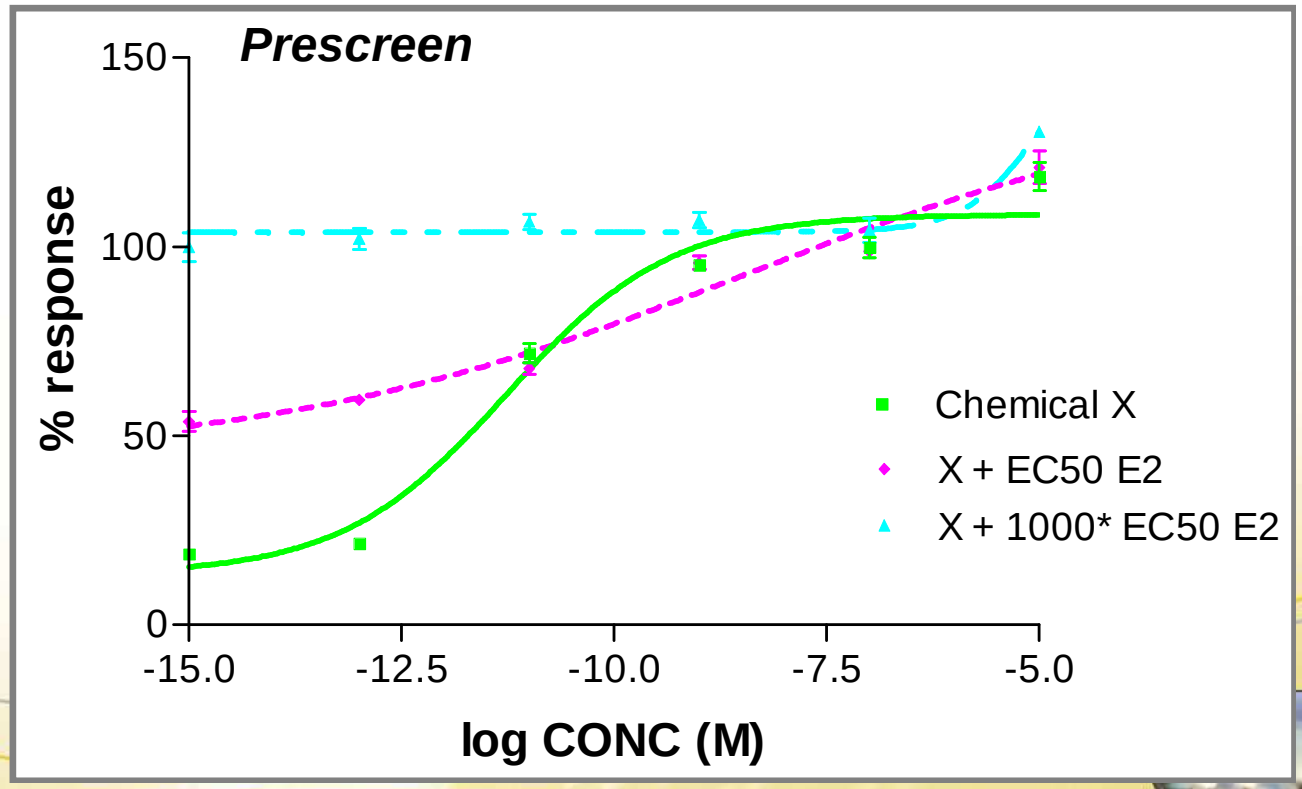
ER transactivation assay

- Stably transfected human breast cancer cell line, developed by INSERM (Balaguer et al, 1999) → ***MELN***



- **Test definition**
  - Mode of action : ER – mediated response
  - Optimise test procedures → SOPs
  - Define acceptance criteria
    - Agonist:
      ⇒ min. induction ratio; EC50 range for 17β-estradiol (PC)
    - Antagonist:
      ⇒ min. inhibition ratio; IC50 range for 4OH-tamoxifen (PC)
  - Include cytotoxic assay: CytoTox-ONE™
- Screening set up

- Test definition
- **Screening set up**

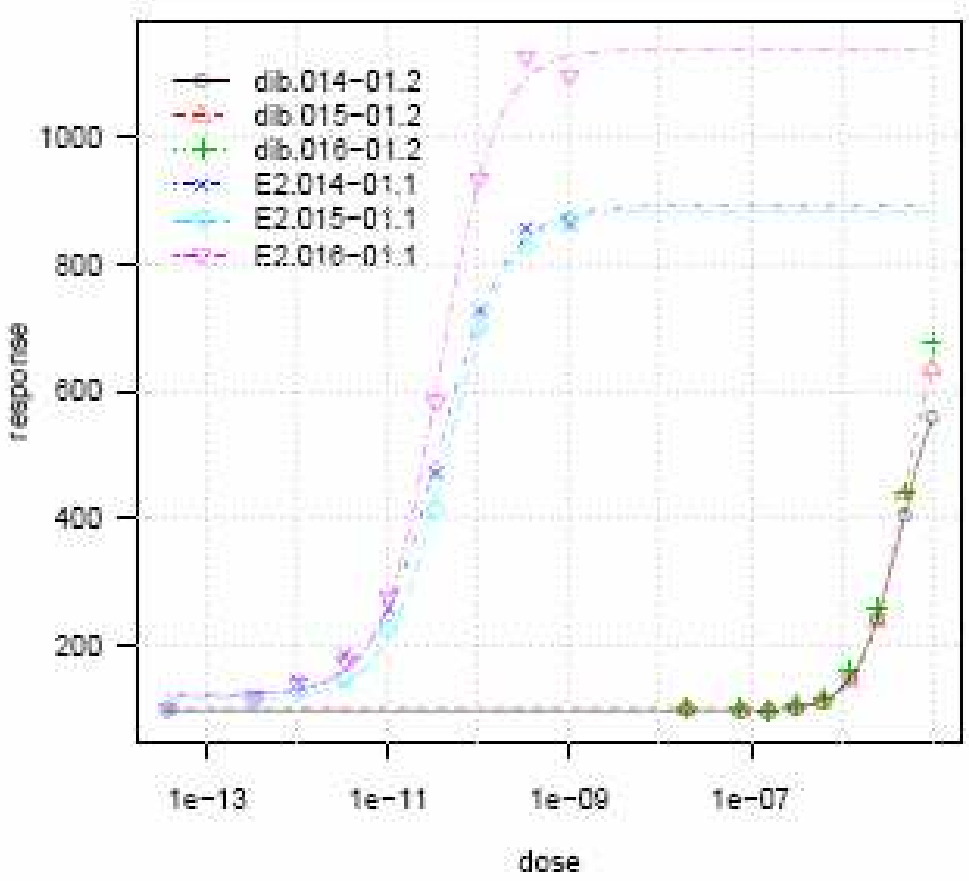


ER transactivation assay

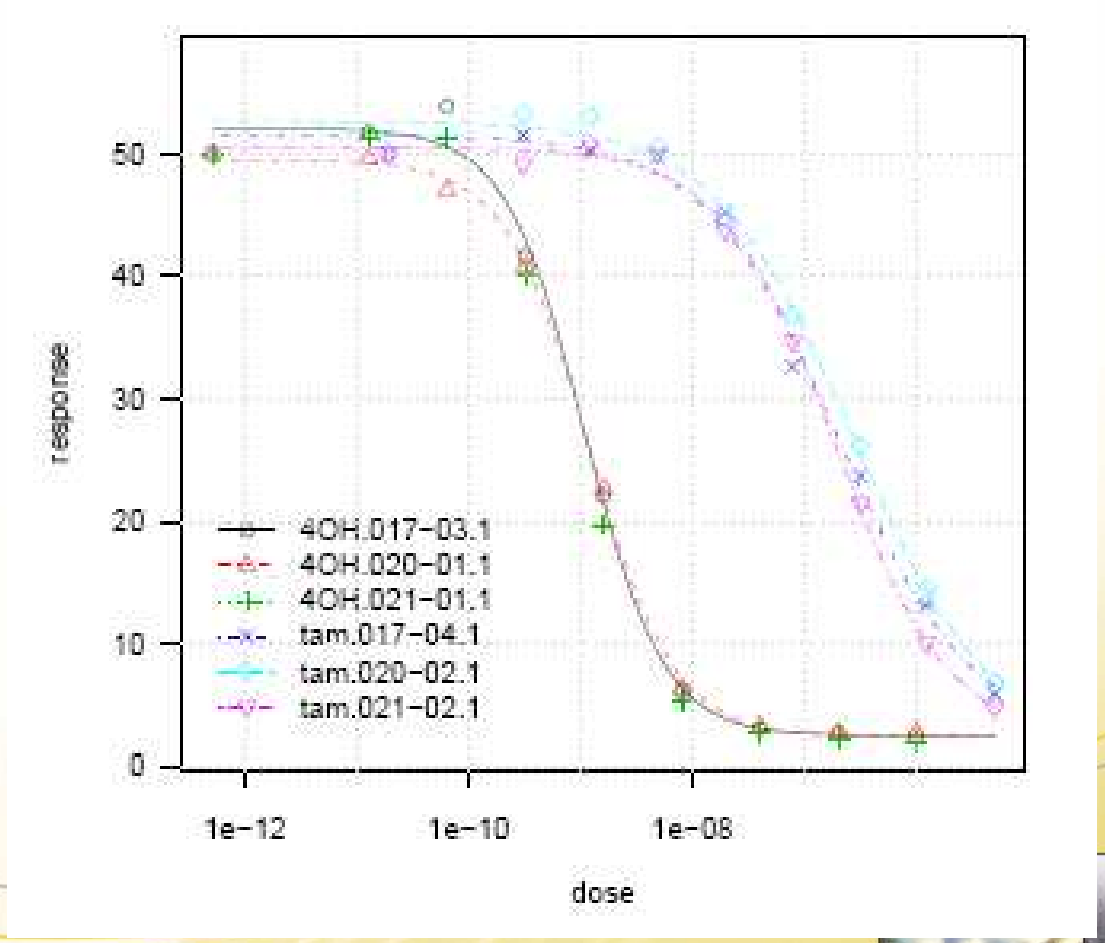
- Test definition
- Screening set up
- **Test chemicals:** selected by EDC task force

Indicative potency	**ER agonist** (12)		**ER-antagonist** (10)	
Strong - moderate	**17β-Estradiol (E_2)** Diethylstilbesterol (DES)	17α-Ethinylestradiol (EE_2) Hexestrol	ICI 182,780 **4OH-Tamoxifen**	Tamoxifen
Mild - weak	Genistein Equol Norethynodrel	di-Butyl paraben Nonylphenol o,p-DDT	Norethynodrel Raloxifene-HCl Genistein	Nonylphenol o,p-DDT
Negative	Corticosterone	Dibutylphtalate (DBP)	Corticosterone	DBP

- **Agonist assay:** dibutylparaben (3 exp.) vs E2



- **Antagonist assay:** tamoxifen (3 exp.) vs OH-Tam



ER transactivation assay

Test chemicals	Agonist assay	
	Er-activation	Mean EC50 (M)
17β-Estradiol (E_2)	+	$4.2\ 10^{-11}$
17α-Ethinylestradiol	+	$2.4\ 10^{-11}$
Diethylstilbesterol	+	$1.7\ 10^{-10}$
Hexestrol	+	$1.0\ 10^{-10}$
Norethynodrel	+	$7.5\ 10^{-9}$
Genistein	+	$8.2\ 10^{-7}$
Nonylphenol	+	$7.4\ 10^{-7}$
opDDT	+	$1.7\ 10^{-6}$
Equol	+	$1.9\ 10^{-6}$
Dibutylparaben	+	$5.4\ 10^{-6}$
Corticosterone	negative	
Dibutylphtalate(DBP)	negative	

Test chemicals	Antagonist assay	
	ER-suppression	Mean IC50 (M)
ICI 182780	+	$6.6\ 10^{-10}$
Raloxifene	+	$2.4\ 10^{-10}$
4OH-Tamoxifen	+	$1.0\ 10^{-9}$
Tamoxifen	+	$2.2\ 10^{-7}$
Norethynodrel	-, activation	
Genistein	-, activation	
Nonylphenol	-, activation	
opDDT	-, activation	
Corticosterone	negative	
Dibutylphtalate(DBP)	negative	

Conclusions and next steps

- Test performance MELN:
  - OK, only 4/80 tests rejected
  - intralab reproducibility : mean CV ± 20% , but limits?
  - classify chemicals on EC50/IC50 → potency class

- Transfer & Interlab reproducibility : under investigation

- Next:
  - predictive value + applicability → relevance
  - comparison to other ER-transactivation tests
  - validation report → OECD

THANK YOU!

- VITO N.V.
  - Pascale Berckmans
  - Clea Vangenechten
  - Katrien Smits
- ECVAM
  - Miriam Jacobs
  - Susanne Bremer

- Partners *in vitro* TA assays
  - Bayer
  - BDS
- Biostatistical evaluation
  - German Cancer Research Center