

# Testing of estrogenic and androgenic reference compounds using 2 versions of the yeast-based YES and YAS assay

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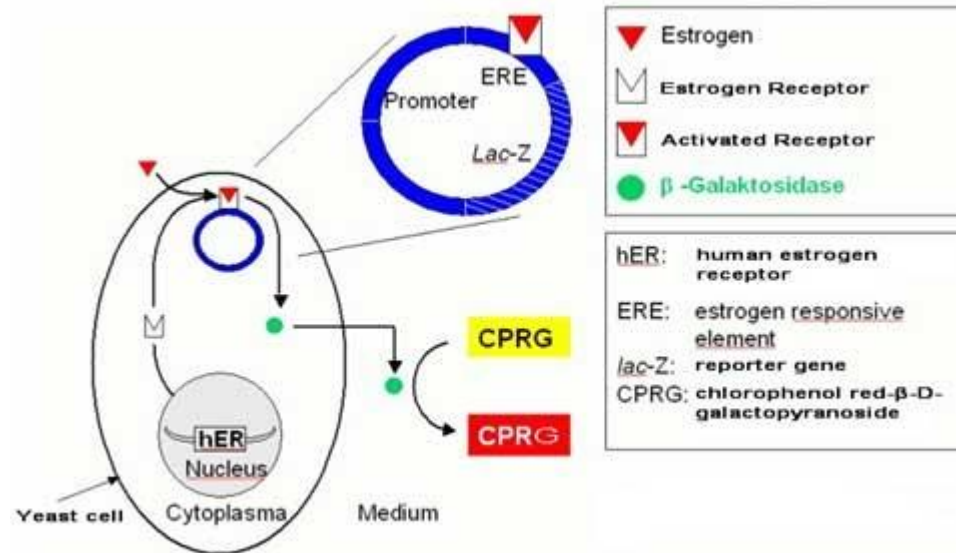
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# XenoScreen and XenoScreen XL YES YAS:

*Routledge, E.J. and Sumpter, J.P. 1996. Environ.Toxicol. Chem. 13; 241-248*

## Common features

- Yeast cells (*Saccharomyces cerevisiae*) transformed with either the human estrogen or androgen receptor
- lacZ ( $\beta$ -galactosidase) based reporter gene with CPRG substrate (yellow  $\rightarrow$  purple)
- Suitable for agonist and antagonist detection
- Short assay time  $\leq$  48 hrs
- Concurrent identification of cytotoxic or growth-arresting effects
- Excel sheet for data evaluation included



# XenoScreen and XenoScreen XL YES YAS: Differences

## XenoScreen (standard version)

- Sample dilutions performed in separate plate
- 48 hrs exposure time
- CPRG present during exposure
- No additional steps before reading

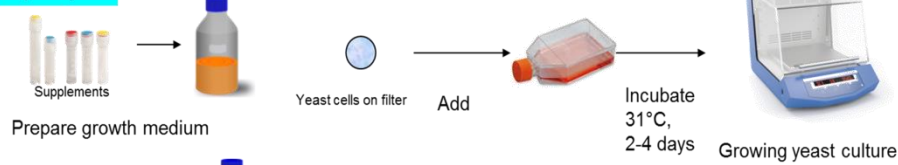
## XenoScreen XL

- Concentrated exposure media allow for minimal dilution of aqueous samples
- Sample dilutions directly in assay plates
- 18 hrs exposure time
- CPRG added after exposure
- Lysis buffer with lyticase to facilitate release of  $\beta$ -galactosidase
- Transfer into fresh plate for final step

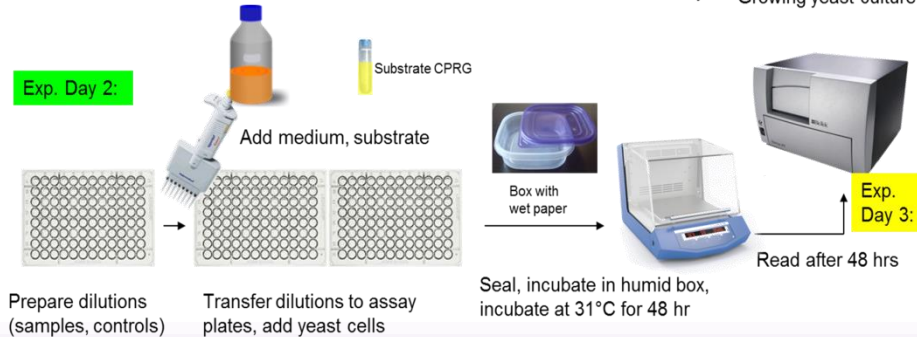
# XenoScreen and XenoScreen XL YES YAS: Differences

## Procedure XenoScreen

Exp. Day 1:

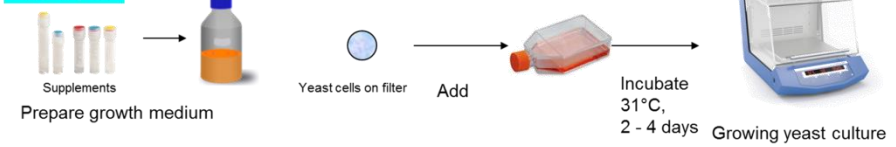


Exp. Day 2:

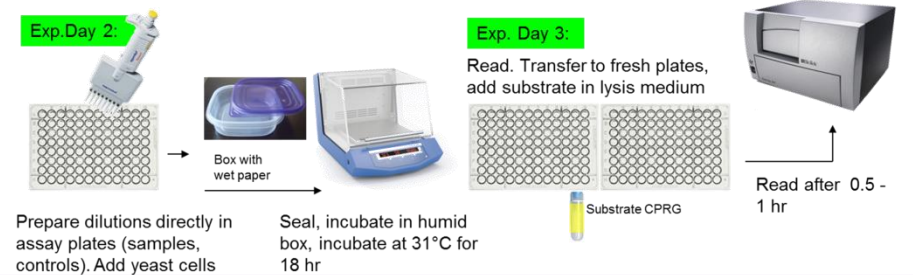


## Procedure XenoScreen XL

Exp. Day 1:



Exp. Day 2:



## Limits of Detection LoD:

Definition: Mean of Solvent Control + 3x SD

<b>17<math>\beta</math>-Estradiol:</b>	<b>XenoScreen:</b>	<b>1.8 x 10<sup>-11</sup> M (4.9 ng/L)</b>
	<b>XenoScreen XL:</b>	<b>4.2 x 10<sup>-12</sup> M (1.1 ng/L)</b>
<b>Dihydrotestosterone:</b>	<b>XenoScreen:</b>	<b>4.0 x 10<sup>-10</sup> M (120 ng/L)</b>
	<b>XenoScreen XL:</b>	<b>2.1 x 10<sup>-10</sup> M (61 ng/L)</b>

## **Estrogen Proficiency Chemicals (YES):**

from *"OECD GUIDELINE 455 FOR THE TESTING OF CHEMICALS: Stably Transfected Human Estrogen Receptor- $\alpha$  Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals*

and from *ICCVAM (2003) ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays*

## **Androgen Proficiency Chemicals (YAS):**

from *"(Draft) OECD GUIDELINE FOR THE TESTING OF CHEMICALS Stably Transfected Human Androgen Receptor- $\alpha$  Transcriptional Activation Assay for Detection of Androgenic Agonist Agonist/Antagonist Activity of Chemicals (Version 2010 Nov. 25)*

and from *ICCVAM (2003) ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays*

## Results with Proficiency Chemicals: YES (estrogenic)

Name	CAS #	Class	Expected response <sup>1)</sup>	Highest tested conc. (M <sup>-1</sup> )	XenoScreen EC50 (M <sup>-1</sup> )	XenoScreen EC10 (M <sup>-1</sup> )	XenoScreen XL EC50 (M <sup>-1</sup> )	XenoScreen XL EC10 (M <sup>-1</sup> )	Remarks
<b>Agonists</b>									
17β-Estradiol	50-28-2	Steroid	positive	1.0 x 10 <sup>-8</sup>	1.4 x 10 <sup>-10</sup>	4.9 x 10 <sup>-11</sup>	5.0 x 10 <sup>-11</sup>	1.4 x 10 <sup>-11</sup>	
Diethylstilbestrol	56-53-1	Cyclic Hydrocarbon	positive	2.5 x 10 <sup>-8</sup>	4.2 x 10 <sup>-10</sup>	2.2 x 10 <sup>-10</sup>	1.6 x 10 <sup>-10</sup>	4.7 x 10 <sup>-11</sup>	
17α-Estradiol	57-91-0	Steroid	positive	1.0 x 10 <sup>-7</sup>	1.1 x 10 <sup>-8</sup>	3.6 x 10 <sup>-9</sup>	1.6 x 10 <sup>-9</sup>	5.1 x 10 <sup>-10</sup>	
meso-Hexestrol	84-16-2	Cyclic Hydrocarbon, Phenol	positive	1.0 x 10 <sup>-7</sup>	2.9 x 10 <sup>-10</sup>	8.0 x 10 <sup>-11</sup>	1.4 x 10 <sup>-10</sup>	2.9 x 10 <sup>-11</sup>	
4-tert-Octylphenol	140-66-9	Phenol	positive	1.0 x 10 <sup>-5</sup>	3.3 x 10 <sup>-6</sup>	1.7 x 10 <sup>-6</sup>	2.6 x 10 <sup>-6</sup>	7.8 x 10 <sup>-7</sup>	
Genistein	446-72-0	Flavonoid, Heterocyclic Cpd.	positive	1.0 x 10 <sup>-5</sup>	3.1 x 10 <sup>-6</sup>	1.1 x 10 <sup>-6</sup>	1.8 x 10 <sup>-7</sup>	5.7 x 10 <sup>-8</sup>	
Bisphenol A	80-05-7	Phenol	positive	1.0 x 10 <sup>-4</sup>	5.1 x 10 <sup>-6</sup>	1.4 x 10 <sup>-6</sup>	2.8 x 10 <sup>-6</sup>	7.8 x 10 <sup>-7</sup>	
Kaempferol	520-18-3	Flavonoid, Heterocyclic Cpd.	positive	1.0 x 10 <sup>-4</sup>	2.9 x 10 <sup>-5</sup>	1.5 x 10 <sup>-5</sup>	7.1 x 10 <sup>-5</sup>	2.0 x 10 <sup>-5</sup>	
Butylbenzyl phthalat	85-68-7	Carocyclic acid, Ester, Phthalic acid	positive	1.0 x 10 <sup>-4</sup>	1.3 x 10 <sup>-4</sup>	4.5 x 10 <sup>-5</sup>	1.3 x 10 <sup>-5</sup>	3.9 x 10 <sup>-6</sup>	
p,p'-Methoxychlor	72-43-5	Halogenated Hydrocarbon	positive	1.0 x 10 <sup>-3</sup>	8.8 x 10 <sup>-5</sup>	2.4 x 10 <sup>-5</sup>	1.9 x 10 <sup>-5</sup>	5.1 x 10 <sup>-6</sup>	
Ethyl paraben	120-47-8	Carboxylic acid, Phenol	positive	1.0 x 10 <sup>-3</sup>	1.6 x 10 <sup>-4</sup>	4.4 x 10 <sup>-5</sup>	1.9 x 10 <sup>-5</sup>	5.1 x 10 <sup>-6</sup>	
Atrazine	1912-24-9	Heterocyclic cpd.	negative	1.0 x 10 <sup>-3</sup>	inactive	inactive	inactive	inactive	
Spirolactone	52-01-7	Lactone, Steroid	negative	1.0 x 10 <sup>-3</sup>	inactive	inactive	inactive	inactive	
Ketoconazole	65277-42-1	Heterocyclic cpd.	negative	1.0 x 10 <sup>-3</sup>	inactive	inactive	inactive	inactive	toxic >1 x 10 <sup>-6</sup>
Reserpine	50-55-5	Heterocyclic cpd., Indole	negative	1.0 x 10 <sup>-3</sup>	inactive	inactive	inactive	inactive	
<b>Antagonists <sup>2)</sup></b>									
4-Hydroxytamoxifen	68047-06-3	Cyclic Hydrocarbon	positive	1.0 x 10 <sup>-5</sup>	1.3 x 10 <sup>-7</sup>		2.7 x 10 <sup>-6</sup>		
<sup>1)</sup> from "OECD GUIDELINE 455 FOR THE TESTING OF CHEMICALS: Stably Transfected Human Estrogen Receptor-α Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals and from ICCVAM (2003) ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays									
<sup>2)</sup> Only EC50 values are given for antagonists, smaller inhibition values are bound to give false positives									

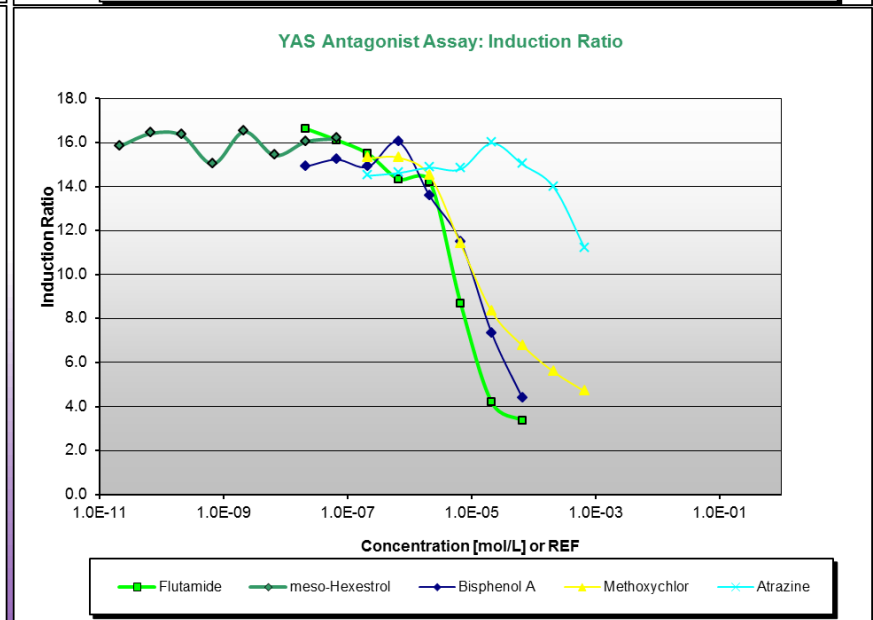
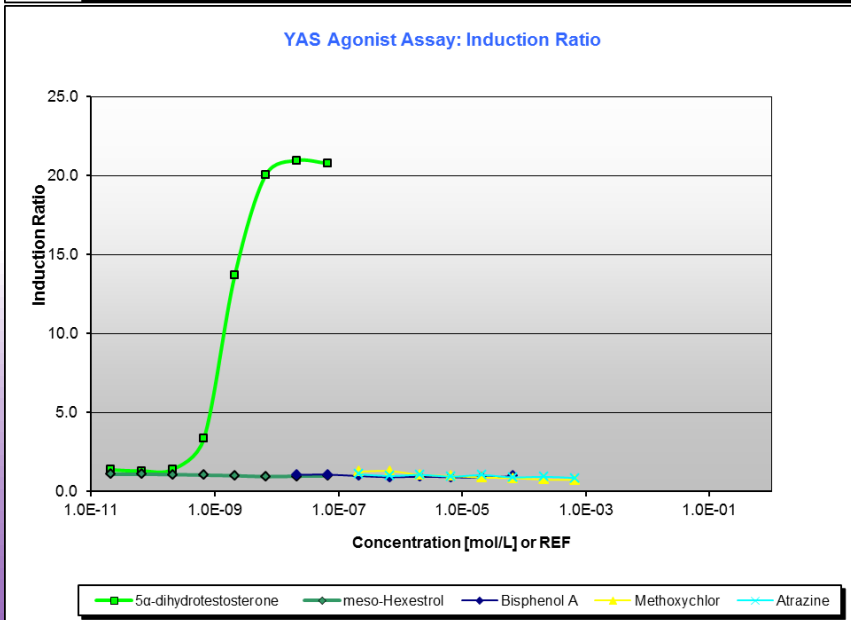
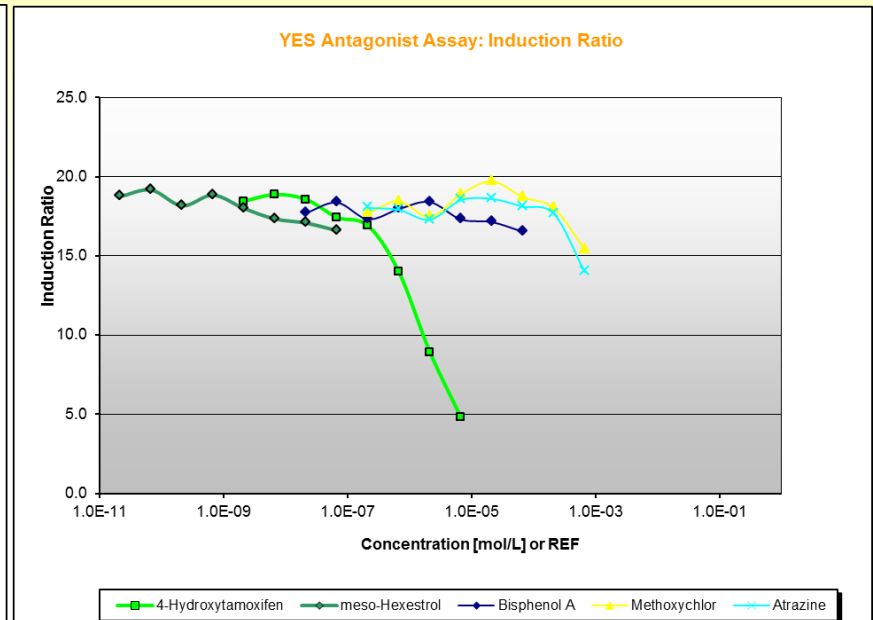
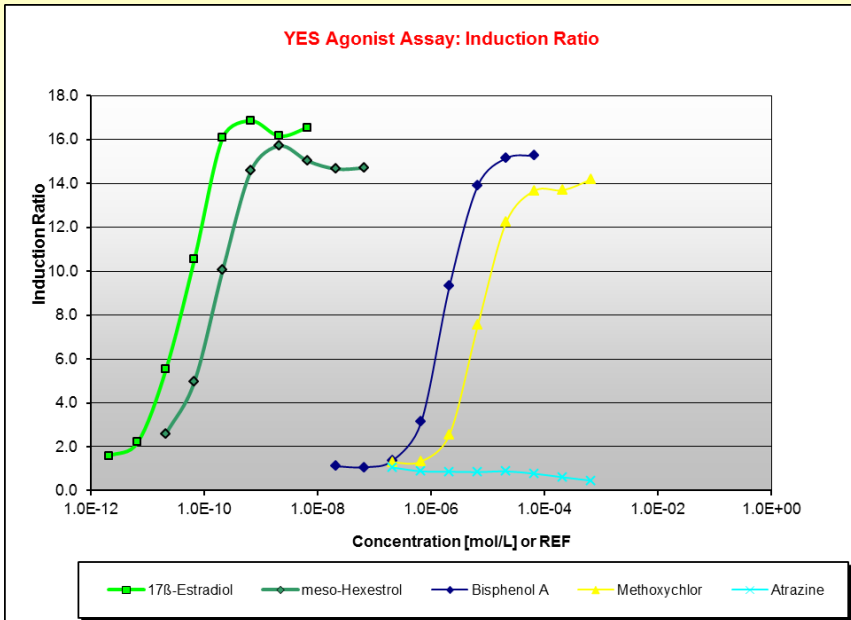


## Results with Proficiency Chemicals: YAS (androgenic)

Name	CAS #	Class	Expected response <sup>1)</sup>	Highest tested conc. (M <sup>-1</sup> )	XenoScreen EC50 (M <sup>-1</sup> )	XenoScreen EC10 (M <sup>-1</sup> )	XenoScreen XL EC50 (M <sup>-1</sup> )	XenoScreen XL EC10 (M <sup>-1</sup> )	Remarks
<b>Agonists</b>									
5α-Dihydrotestosterone	521-18-6	Steroid, nonphenolic	positive	1 x 10 <sup>-6</sup>	4.3 x 10 <sup>-9</sup>	6.5 x 10 <sup>-10</sup>	2.0 x 10 <sup>-9</sup>	4.7 x 10 <sup>-10</sup>	
Testosterone	58-22-0	Steroid, nonphenolic	positive	1 x 10 <sup>-5</sup>	4.5 x 10 <sup>-9</sup>	7.5 x 10 <sup>-10</sup>	2.9 x 10 <sup>-9</sup>	5.6 x 10 <sup>-10</sup>	
17α-Methyltestosterone	58-18-4	Steroid, nonphenolic; Androstene	positive	1 x 10 <sup>-7</sup>	9.4 x 10 <sup>-9</sup>	1.7 x 10 <sup>-9</sup>	4.6 x 10 <sup>-9</sup>	8.9 x 10 <sup>-10</sup>	
Medroxyprogesteroneacetate	71-58-9	Polycyclic hydrocarbon	positive	1 x 10 <sup>-6</sup>	2.9 x 10 <sup>-8</sup>	4.7 x 10 <sup>-9</sup>	1.5 x 10 <sup>-8</sup>	2.9 x 10 <sup>-9</sup>	
Progesterone	57-83-0	Steroid, nonphenolic; Pregnenedione	positive	1 x 10 <sup>-5</sup>	4.6 x 10 <sup>-8</sup>	8.2 x 10 <sup>-9</sup>	8.9 x 10 <sup>-9</sup>	2.2 x 10 <sup>-9</sup>	
4-Androstenedione	63-05-8	Steroid, nonphenolic	positive	1 x 10 <sup>-5</sup>	4.2 x 10 <sup>-7</sup>	1.4 x 10 <sup>-8</sup>	1.2 x 10 <sup>-8</sup>	3.0 x 10 <sup>-9</sup>	
Spirolactone	52-01-7	Steroid, nonphenolic; Pregnene lactone	positive	1 x 10 <sup>-3</sup>	1.4 x 10 <sup>-5</sup>	1.3 x 10 <sup>-6</sup>	2.6 x 10 <sup>-6</sup>	6.7 x 10 <sup>-7</sup>	
Dexamethasone	50-02-2	Steroid, nonphenolic	positive	1 x 10 <sup>-3</sup>	>1 x 10 <sup>-3</sup>	>1 x 10 <sup>-3</sup>	1.4 x 10 <sup>-3</sup>	3.1 x 10 <sup>-4</sup>	
Flutamide	13311-84-7	Amide; Anilide; Nitrobenzene	negative	1 x 10 <sup>-4</sup>	inactive	inactive	inactive	inactive	
4-tert-Octylphenol	140-66-9	Alkylphenol; Phenol	negative	1 x 10 <sup>-3</sup>	inactive	inactive	inactive	inactive	toxic > 1 x 10 <sup>-5</sup>
Bisphenol A	80-05-7	Diphenylalkane; Bisphenol; Phenol	negative	1 x 10 <sup>-3</sup>	inactive	inactive	inactive	inactive	
Di(2-ethylhexyl)phthalate	117-81-7	Phthalate	negative	1 x 10 <sup>-3</sup>	inactive	inactive	inactive	inactive	
<b>Antagonists <sup>2)</sup></b>									
Hydroxyflutamide	52806-53-8	Amide; Anilide; Nitrobenzene	positive	1 x 10 <sup>-4</sup>	1 x 10 <sup>-5</sup>	-	6 x 10 <sup>-6</sup>	-	
Bisphenol A	80-05-7	Diphenylalkane; Bisphenol; Phenol	positive	1 x 10 <sup>-3</sup>	3 x 10 <sup>-4</sup>	-	4 x 10 <sup>-5</sup>	-	
Flutamide	13311-84-7	Amide; Anilide; Nitrobenzene	positive	1 x 10 <sup>-4</sup>	2.0 x 10 <sup>-5</sup>	-	1.2 x 10 <sup>-5</sup>	-	
Spirolactone	52-01-7	Steroid, nonphenolic; Pregnene lactone	positive	1 x 10 <sup>-3</sup>	>1 x 10 <sup>-3</sup> , bi-phasic	-	inactive	-	Agonist
Procymidone	32809-16-8	Organochlorine; Cyclic imide	positive	1 x 10 <sup>-3</sup>	5.0 x 10 <sup>-6</sup>	-	2.0 x 10 <sup>-6</sup>	-	
Progesterone	57-83-0	Steroid, nonphenolic; Pregnenedione	positive	1 x 10 <sup>-5</sup>	inactive	-	inactive	-	Strong agonist
Vindozoline	50471-44-8	Organochlorine; Cyclic imide; Carbamate	positive	1 x 10 <sup>-3</sup>	2.8 x 10 <sup>-5</sup>	-	9 x 10 <sup>-6</sup>	-	
Medroxyprogesteroneacetate	71-58-9	Steroid, nonphenolic; Polycyclic hydrocarbon	negative	1 x 10 <sup>-6</sup>	inactive	-	inactive	-	
<sup>1)</sup> from "(Draft) OECD GUIDELINE FOR THE TESTING OF CHEMICALS Stably Transfected Human Androgen Receptor-α Transcriptional Activation Assay for Detection of Androgenic Agonist/Antagonist Activity of Chemicals (Version 2010 Nov. 25) and from ICCVAM (2003) ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays									
<sup>2)</sup> Only EC50 values are given for antagonists, smaller inhibition values are bound to give false positives									

# Conclusions:

- Both versions of the XenoScreen assay (standard and XL) are able to correctly identify strong to weak estrogenic and androgenic proficiency chemicals. Both activating and inhibiting activities can be measured. The 2 only exceptions in the YAS antagonist assay can be explained by dominance of of the agonistic effect in dual-mode compounds.
- No false positives with negative proficiency chemicals
- Both versions are easy to handle, have a good sensitivity and reproducibility.



Thank you!