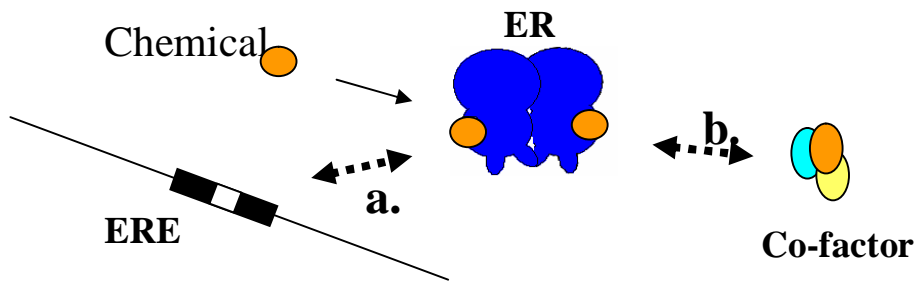
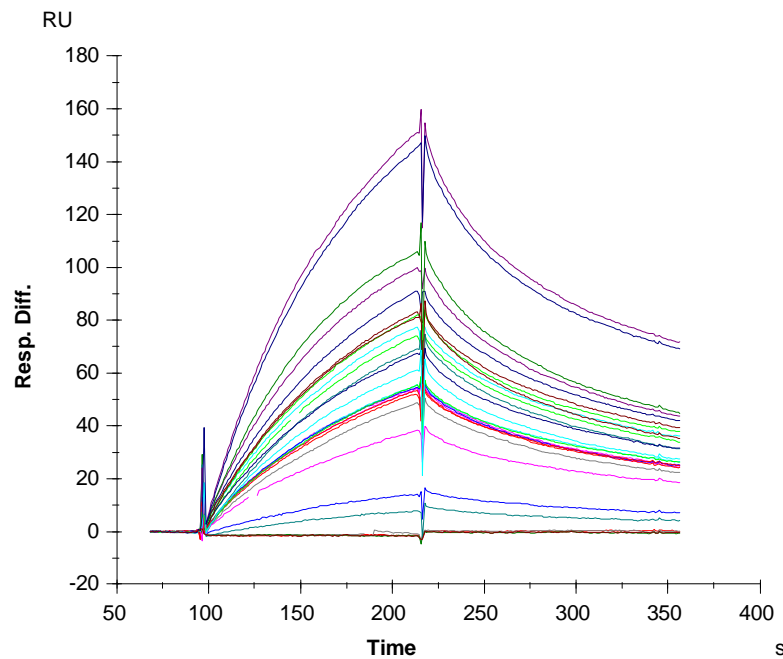


# SPR-HTPS assay

data correction was contracted via  
 BIACORE K.K.

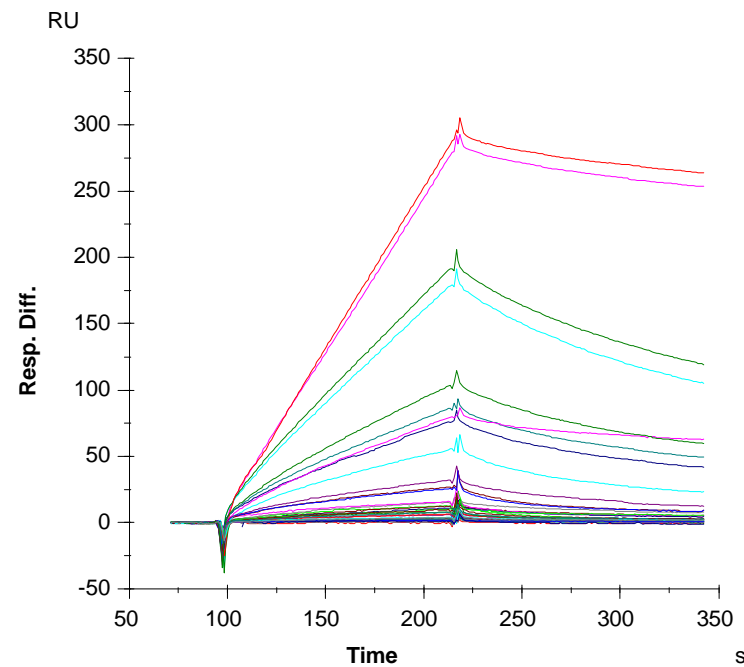


## a. ER-ERE interaction



No.	Test No.	CAS No.	Name	ERE assay		TIF assay	
				ERE 1	ERE 2	TIF 1	TIF 2
1	101-01	000050-28-2	Estradiol	H	H	H	H
2	101-02	000057-91-0	ESTRA-1,3,5(10)-TRIENE-3,17-DIOL (17ALPHA)	H	H	H	H
3	101-03	000053-16-7	Estrone	H	H	H	L
4	101-05	000057-63-6	19-Nor-17-alpha-pregna-1,3,5(10)-trien-20-yne-3,17-diol	H	H	H	H
5	104-01	000362-05-0	Estra-1,3,5(10)-triene-2,3,17-beta-triol	H	H	H	H
6	104-03	000362-07-2	ESTRA-1,3,5(10)-TRIENE-3,17-DIOL, 2-METHOXY-, (17.BETA.)-	L	L	L	L
7	106-07	000068-22-4	19-Nor-17-alpha-pregn-4-en-20-yn-3-one, 17-hydroxy-	L	L	L	L
8	106-09	000063-05-8	Androst-4-ene-3,17-dione	N	N	N	N
9	107-04	000057-83-0	Progesterone	N	N	N	N
10	211-10	000501-24-6	3-PENTADECYLPHENOL	N	N	N	N
11	221-06	005153-25-3	Benzoic acid, 4-hydroxy-, 2-ethylhexyl ester	N	L	L	L
12	242-05	001034-01-1	Gallic acid, octyl ester	N	N	N	N
13	321-09	006807-17-6	4,4'-(1,3-DIMETHYLBUTYLIDENE)BISPHENOL	L	L	N	N
14	321-11	027955-94-8	Phenol, 4,4'-(4-ethylidene)di-	N	N	N	N
15	321-12	000081-92-5	BENZENEMETHANOL, 2-[BIS(4-HYDROXYPHENYL)METHYL]-	L	L	N	N
16	321-18	000081-90-3	o-Toluic acid, alpha, alpha-bis(p-hydroxyphenyl)-	N	N	N	N
17	321-27	000978-86-9	4-(Triphenylmethyl)phenol	L	L	N	N
18	321-28	062625-31-4	Phenol, 4,4'-(3H-1,2-benzoxathiol-3-ylidene)bis 3-methyl-, S,S-dioxide, monosod	N	N	N	N
19	321-30	005384-21-4	PHENOL, 4,4'-METHYLENEBIS[2,6-DIMETHYL-	L	L	N	N
20	321-31	005613-46-7	2,6-Xylenol, 4,4'-isopropylidenedi-	L	L	L	L
21	331-02	000084-16-2	Phenol, 4,4'-(1,2-diethylethylene)di-, meso-	H	H	H	H
22	331-03	000084-17-3	Phenol, 4,4'-(diethylenedioxy)di-	L	H	H	H
23	332-02	56-53-1	diethylstilbestrol	H	H	H	H
24	361-06	006893-02-3	Alanine, 3-(4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl)-, L-	N	N	N	N
25	391-11	000500-58-9	NORDIHYDROQUAIARETIC ACID	N	N	N	N
26	391-12	023238-51-2	Benzyl alcohol, p-hydroxy-alpha-(1-(p-hydroxyphenyl)amino)ethyl-, hydrochlor	N	N	N	N
27	391-16	001050-28-8	L-Tyrosine, N-L-tyrosyl-	N	N	N	N
28	491-16	000145-50-6	1(4H)-Naphthalenone, 4- .alpha.-(4-hydroxy-1-naphthyl)benzylidene -	L	L	N	N
29	501-02	000446-72-0	Genistein	L	L	L	L
30	501-01	000080-05-7	Bisphenol A	L	L	N	N

## b. ER-LxxLL interaction



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## Current status of *in vitro* test methods for potential EDCs

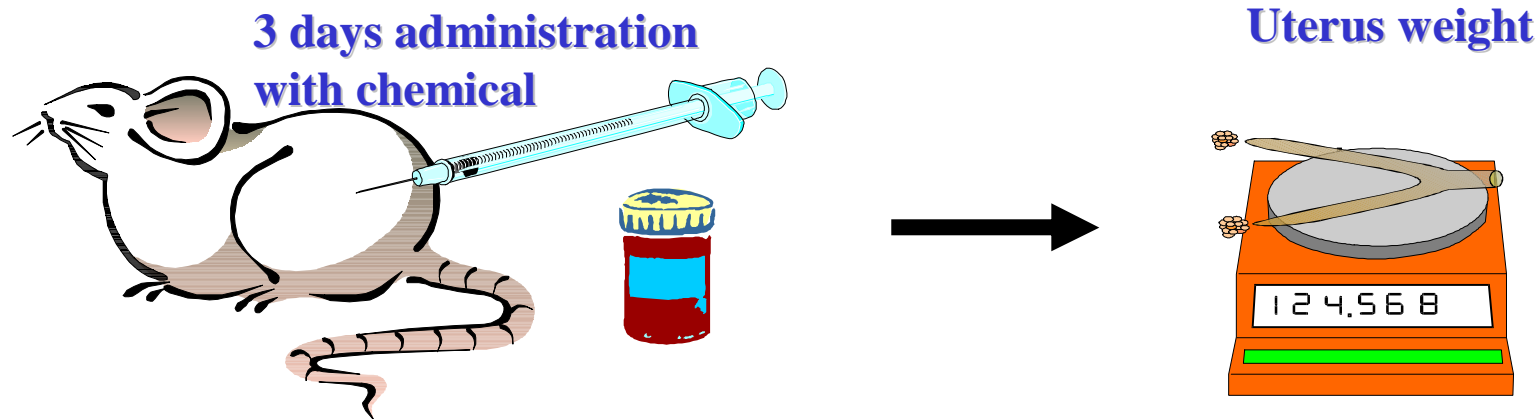
Assay method	Target protein/cell	status	No. of tests	Future plan
<b>Receptor binding</b>	ER	<b>Under validation</b>	ca. 1,600	<b>Data accumulation Submission for OECD guideline</b>
	AR	<b>Under validation</b>	ca. 900	<b>Data accumulation Submission for OECD guideline</b>
	TR	<b>Under development</b>	<20	-
<b>Reporter gene assay</b>	ER(agonist)	<b>Under validation</b>	ca. 1,600	<b>Data accumulation Submission for OECD guideline</b>
	AR(agonist/antagonist)	<b>Under validation</b>	ca. 900	<b>Data accumulation Submission for OECD guideline</b>
	TR(agonist/antagonist)	<b>Under development</b>	<20	-
<b>Aromatase assay</b>	KGN cell	<b>Under validation</b>	ca. 570	<b>Data accumulation</b>
<b>Steroidgenesis</b>	H295R	<b>Under development</b>	-	<b>Submission for OECD guideline</b>

# Level 3 testing

- Uterotrophic assay
- Hershberger assay

# Outline of Uterotrophic assay

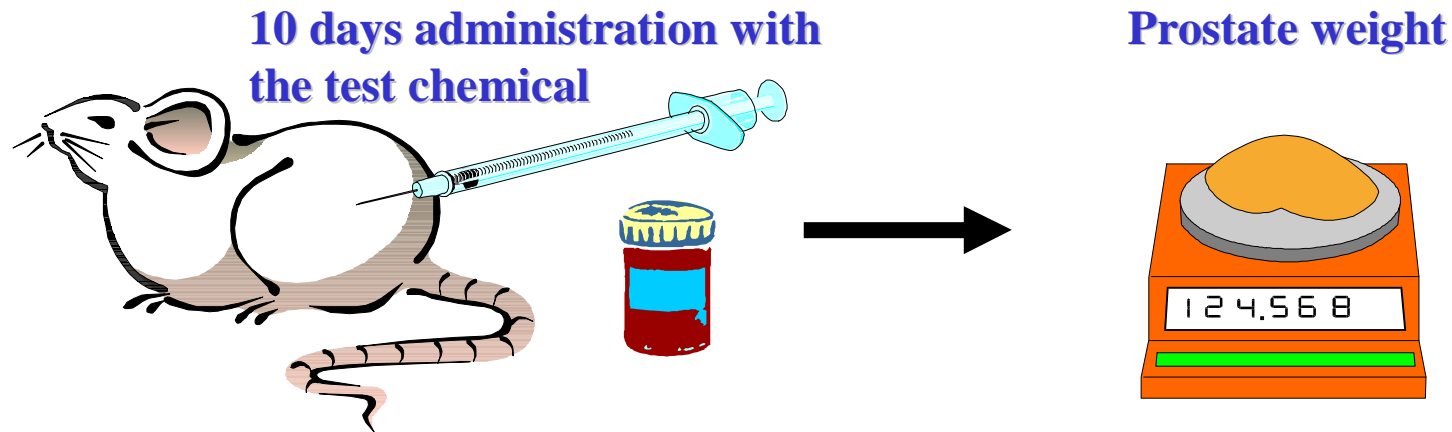
- Estrogenic chemicals induce uterus enlargement. Based on this principle, rats are administered with chemicals for three days and then uterus weights are measured as an endpoint.



**Test chemical would be defined as “estrogenic” when uterus weight of the test group is significantly increased compared with that of control.**

# Outline of Hershberger assay

- Androgenic chemicals induce an enlargement of male accessory sex organs such as the prostate. Based on this principle, castrated rats are administered with test chemicals for 10 days and then the prostate or other accessory sex organs weights will be measured as an endpoint.



**Test chemical would be defined as “androgenic” when accessory sex organs weight of the test group are significantly increased compared with that of control.**

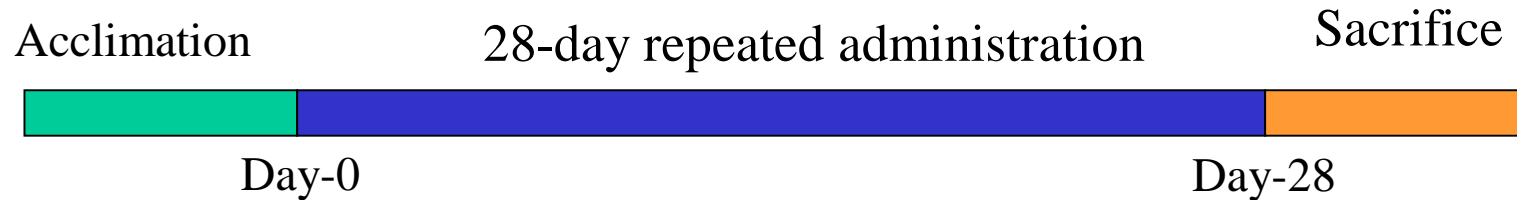
## Current status of the Level 3 testing

- The uterotrophic assay has completed its peer review process for inclusion in OECD guidelines.
- The peer review process of the Hershberger assay is currently in progress.

# Level 4 testing

- Enhanced OECD 407 (endpoints based on endocrine mechanisms)

# Enhanced OECD TG407 study



**Based on the OECD TG407 and reinforced to detect endocrine effect of chemicals by addition of supplemental endpoints**

Enhanced items for detection of endocrine effects:

- Serum hormone levels T3, T4, TSH, etc.
- Sperm analysis
- Estrus cycle etc.



## Current status of the Level 4 testing

- Enhanced TG407 study was agreed to progress toward alternative to current TG407, and the international peer review process has just started.

# Level 5 testing

- Two-generation study
- *In utero* and lactationary exposure study
- Other trial