EDA-EMERGE SC2 – 30.01.2013

In vitro bioassays for EDC detection

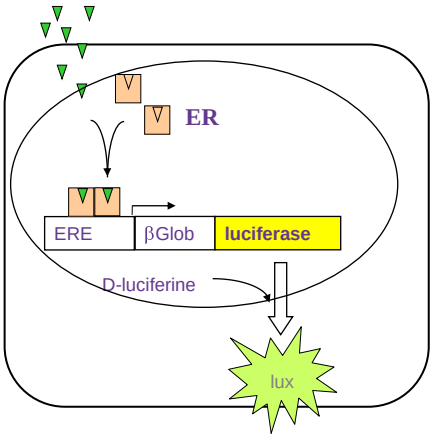
Selim Aït-Aïssa

INERIS

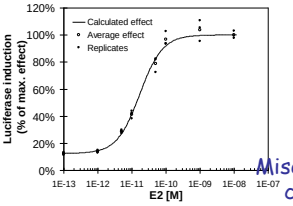
Outline

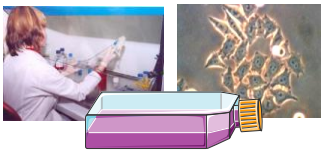
1. Principles of in vitro assays and TEFs
2. Sources of variation between assays (cell contexts, cross-species…)
3. Analysis of complex mixtures : how to accurately quantify TEQs ?

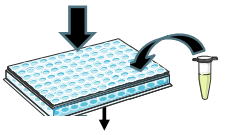
Bioassay : reporter cell line



Stably transfected cell line

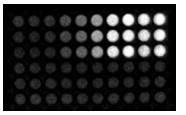






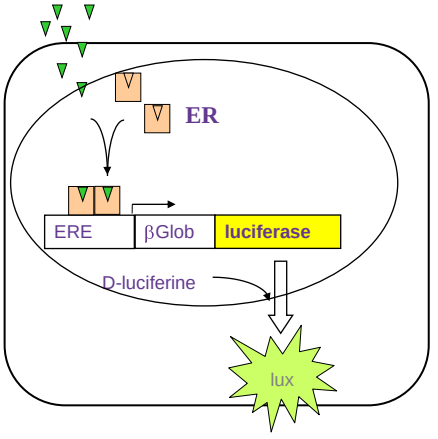
Mise en présence de différentes concentrations de toxiques

16 h

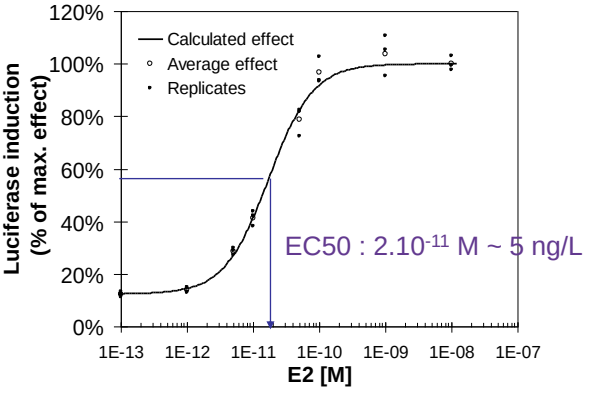


Mesure de la luminescence

Bioassay : reporter cell line



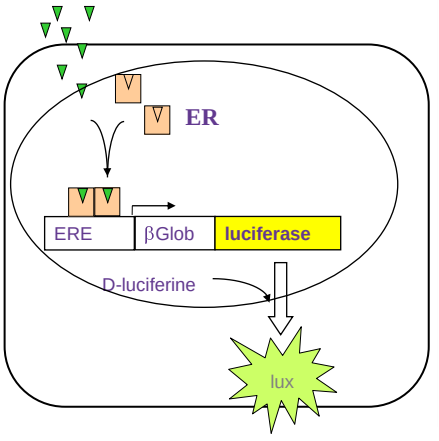
Stably transfected cell line



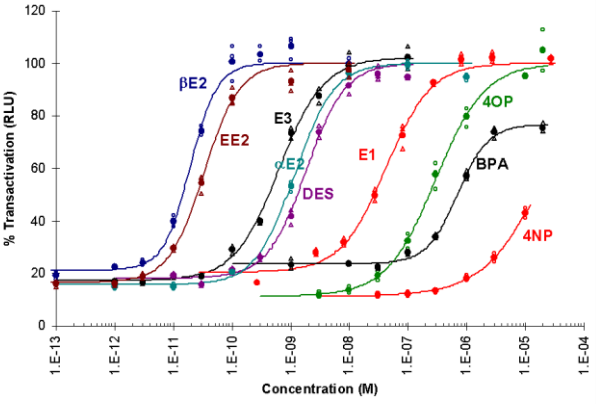
EC50 = concentration induisant 50 % de l'effet maximum

EDA-EMERGE SC2 - 30/01/13

Bioassay : reporter cell line



Stably transfected cell line



Relative potency

$EEF = EC50_{E2} / EC50_{test\ chemical}$

(EEF = Estradiol Equivalent factor)

Examples of existing *in vitro* reporter gene assays for estrogenicity

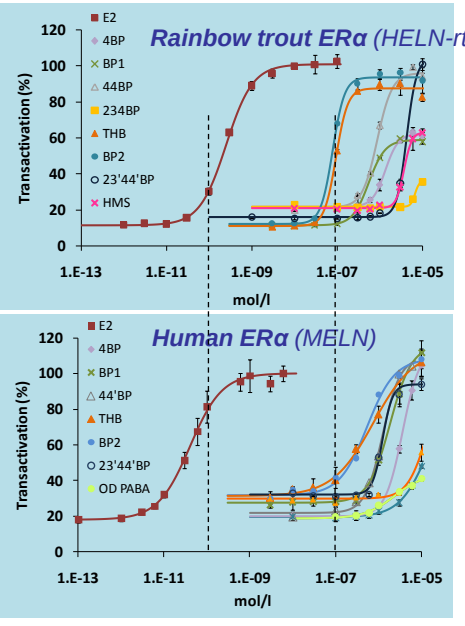
Assays	Species	Cell line	Tissue	Endpoint
ER-CALUX	Human	T47D	Mammary	Luc
T47D-kbLuc	Human	T47D	Mammary	Luc
MELN	Human	MCF-7	Mammary	Luc
HeLa9903	Human	HeLa	Cervix	Luc
BG1Luc	Human	BG1	Ovary	Luc
HELN-hER	Human	HeLa	Cervix	Luc
HELN-rtER, -zfER	Human/Fish	HeLa	Cervix	Luc
RTG-2	Rainbow trout	RTG-2	Gonad	Luc
PELN-rtER	Topminnow	PLHC-1	Liver	Luc
ZELH-zfERs	Zebrafish	ZFL	Liver	Luc
U251-MG-zfER	Human/ZF	U251-MG	Radial Glial cells	Luc
YES (hER, rtER)	Yeast			B-gal
BLYES	Yeast			Luc

Factors that can modulate ER-mediated signal

- Cellular context : different co-transcriptional factors
- Cellular context : metabolic capacities
- Cellular context : cross-talk between ER and other NR-mediated signaling pathways (e.g. AhR)
- Cross-species differences : different receptor structures (sequence) or isoforms that can explain different affinities for chemical ligands, or even specificity for some NRs

- 3 examples: fish *vs* human cell models

Cross-species differences : fish- *vs*. human-based assays



Rainbow trout vs human ERα

⇨Different dose-reponse behavior : partial/full agonists

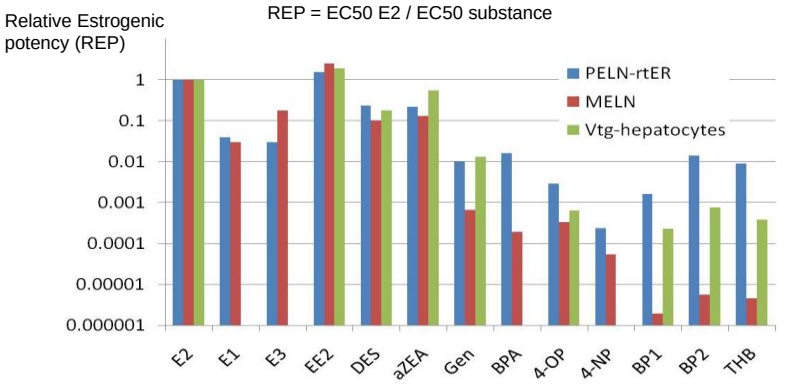
⇨ Different sensitivities :

E2 : hER > rtER

BP2, THB : hER ≤ rtER

⇨ Different relative potency (TEF)

Comparison of human (MELN) and fish (PELN et Vtg-PRTH) assays for estrogenicity



Cosnefroy et al, Toxicol in vitro, 2009

⇨ Higher REPs in fish

⇨ Fish-based assays more relevant to assess estrogenic hazard in this organism

Zebrafish ER

3 estrogen receptors in ZF: zfERα, zfERβ1 and zfERβ2
2 in humans : hERα and hERβ

Differential expression of zfERs depending of the tissue, gender, developmental stage → different function/role

Difference in structures (LBD) → affinity of chemical ligands

Need to assess zfER selectivity when evaluating estrogenic action of chemicals in zebrafish

Sequence homology between zfERs and hERs

A

	zfERα	zfERβ1	zfERβ2	hERα
zfERα	100			
zfERβ1	40.5	100		
zfERβ2	39.4	51.5	100	
hERα	47.1	39	37.8	100
hERβ	40.9	44.4	46.8	37.6

B

	zfERα	zfERβ1	zfERβ2	hERα
zfERα	100			
zfERβ1	10.8	100		
zfERβ2	12.5	26.6	100	
hERα	8.4	9.6	8.4	100
hERβ	13.2	8.9	13.2	8.4

	zfERα	zfERβ1	zfERβ2	hERα
zfERα	100			
zfERβ1	82.1	100		
zfERβ2	83.3	90.5	100	
hERα	91.7	82.1	84.5	100
hERβ	83.3	91.7	90.5	82.1

	zfERα	zfERβ1	zfERβ2	hERα
zfERα	100			
zfERβ1	11.1	100		
zfERβ2	15.7	7.4	100	
hERα	19.6	11.1	12.2	100
hERβ	19.6	5.6	6.7	20.4

	zfERα	zfERβ1	zfERβ2	hERα
zfERα	100			
zfERβ1	50.3	100		
zfERβ2	48.3	61.8	100	
hERα	55.2	49.3	48.9	100
hERβ	49.7	56.8	61.4	

In vitro activation of zfERs by (xeno)estrogens

	ZELH-zfERα				ZELH-zfERβ1				ZELH-zfERβ2			
	EC_{50} (nM)	SD	*n*	REP	EC_{50} (nM)	SD	*n*	REP	EC_{50} (nM)	SD	*n*	REP
E2	0.20	0.05	15	1	0.03	0.01	14	1.00	0.05	0.02	15	1.00
E1	1.80	0.80	5	0.11	0.48	0.10	4	0.06	0.48	0.18	6	0.11
E3	2.04	0.91	3	0.10	2.00	1.69	4	0.01	1.24	0.55	10	0.04
EE2	0.13	0.04	4	1.52	0.03	0.01	4	0.82	0.03	0.00	3	1.53
HEX	0.87	0.39	6	0.24	1.62	0.73	4	0.02	0.89	0.51	7	0.06
DES	0.06	0.02	4	3.48	0.09	0.06	5	0.30	0.06	0.02	6	0.85
α-Zea	68	39.2	4	3.0E-03	127	82	3	2.1E-04	265	118	5	1.9E-04
α-Zee	144	105	6	1.4E-03	154	144	6	1.8E-04	262	182	5	1.9E-04
β-Zee	224	151	4	9.1E-04	5212	1614	3	5.3E-06	1893	2118	6	2.7E-05
Gen	277	182	7	7.4E-04	553	319	6	4.9E-05	355	298	6	1.4E-04
BP1	2195	1395	5	9.3E-05	w.e.		3		3859	1617	4	1.3E-05
BP2	1084	780	4	1.9E-04	2216	909	4	1.2E-05	1477	694	4	3.4E-05
BP3	n.e.		3		n.e.		2		w.e.		3	
THB	1062	429	4	1.9E-04	3718	757	3	7.4E-06	3902	1285	4	1.3E-05
BPA	1167	947	7	1.8E-04	w.e.		3		2106	748	4	2.4E-05
4tOP	332	111	3	6.2E-04	n.e.		3		242	76	3	2.1E-04
o,p'-DDT	1359	831	3	1.5E-04	w.e.		3		217	41	3	2.3E-04

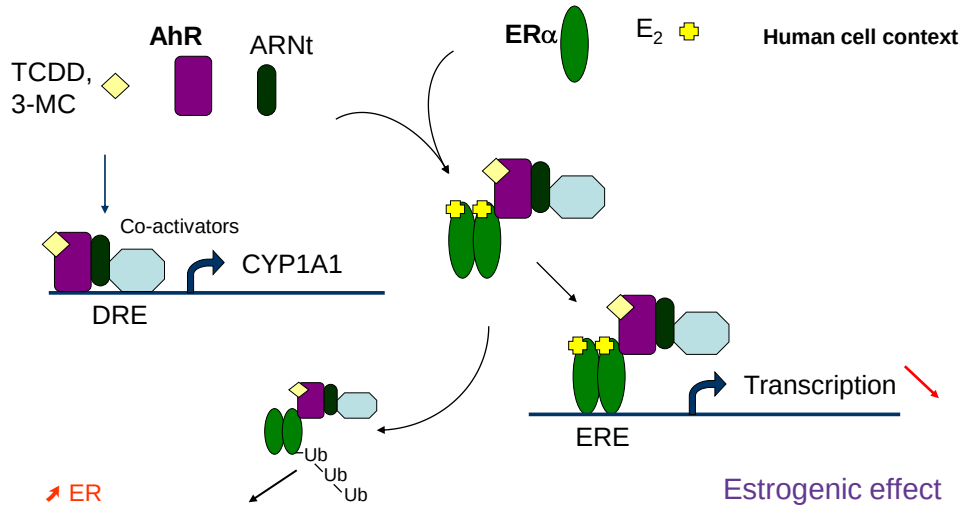
Cosnefroy et al, Toxicol Sci, 2012

Alpha / beta selectivity, e.g.:

steroids have higher affinity for zfER beta isoforms

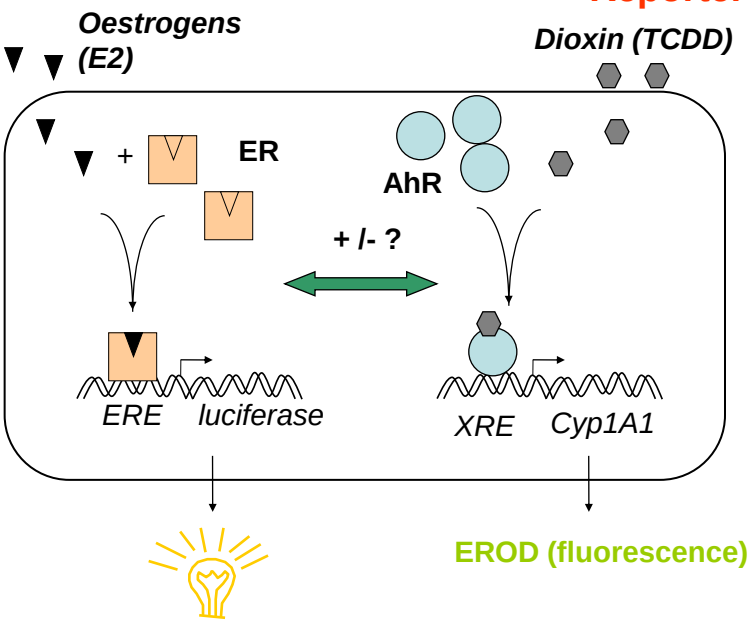
BPs, BPA more affine for alpha isoform

ER-AhR cross-talk

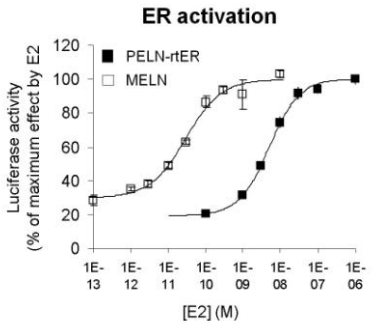


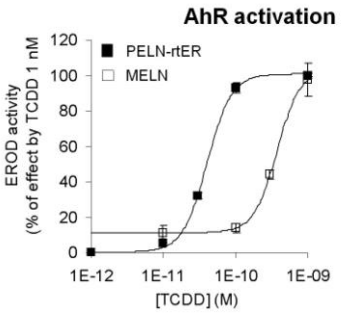
Wormke et al, *Mol. Cell Biol.* 2003; Ohtake et al, *Nature*, 2003, 2007

Reporter cell lines

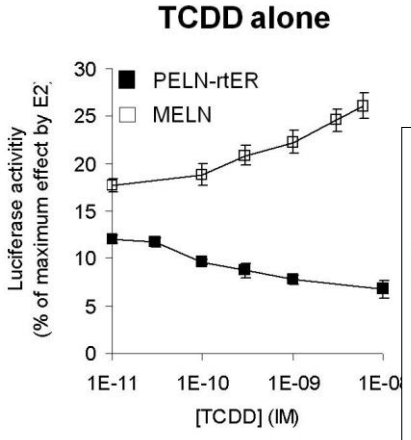


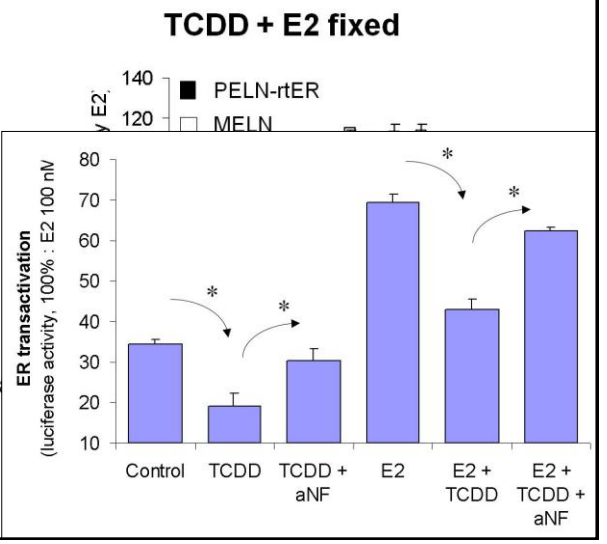
Fish vs human ER : different sensitivity to reference ER and AhR ligands





Different effects of dioxin (TCDD) on ER activation in fish and human assays





(Anti)estrogenic and dioxin-like activity of PAHs

	Fish (PELN-rtER)		Human (MELN)	
PAHs	AhR	ER	AhR	ER
CHR	+++	-	+++	↗↗
DBA	+++	↘	+	↗
BkF	+++	↘	+	↗
3-MC	+++	-	+++	↗↗
BaP	+++	↘	+++	↗↗
BaA	++	↘	++	↗
PYR	+/-	-	+/-	-
FLU	-	-	-	-
BAP-3OH	+/-	-	+++	↗↗
FLU-2OH	+/-	-	+/-	↗
FLU-9OH	+/-	-	+/-	-
PYR-OH	+/-	-	+/-	↗

+ : active ↗ : induction

- : non active ↘ : inhibition

Between-bioassay differences : implication for complex mixture assessment

Differences in model sensitivity : relative potencies of chemical will vary between assays

Developing and validating fish-based assays for environmental monitoring is a major task

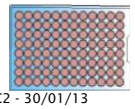
Different TEF from different bioassays : recommendation : well characterize your assay with individual chemicals before use for complex mixture assessment

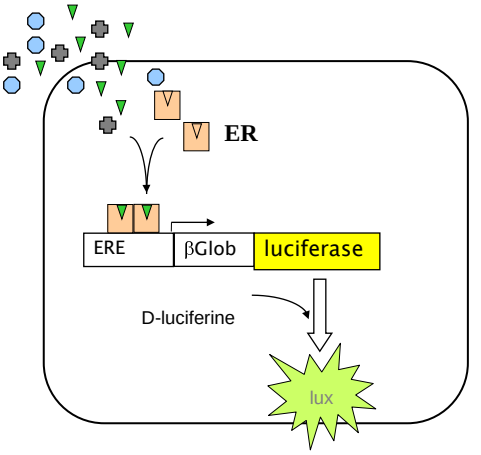
Biodetection of EDCs in complex mixtures

Sediment Effluent

Organic extraction (SPE, ASE…)

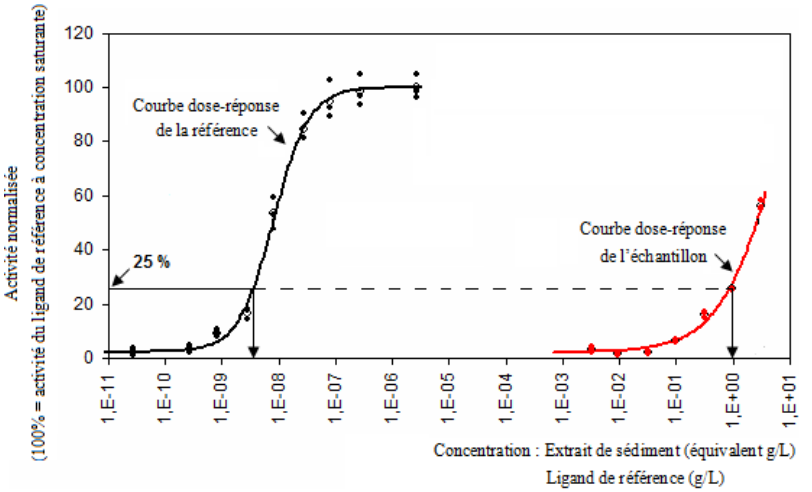
Organic extract





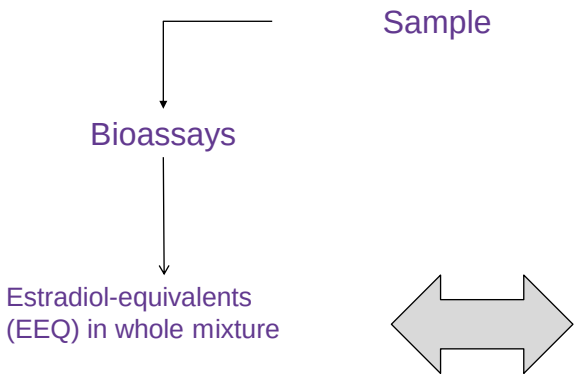
=> Detection of all ligands present in the complex mixture

Determination of biological Toxic-Equivalents (TEQ)



$$TEQ = \frac{CE_{25}\ (\text{substance de réference})}{CE_{25}\ (\text{échantillon})}$$

Mass balance analysis: biological- vs chemical-TEQs

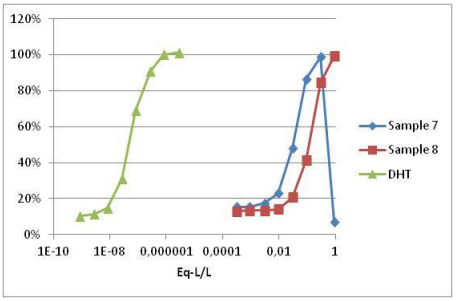


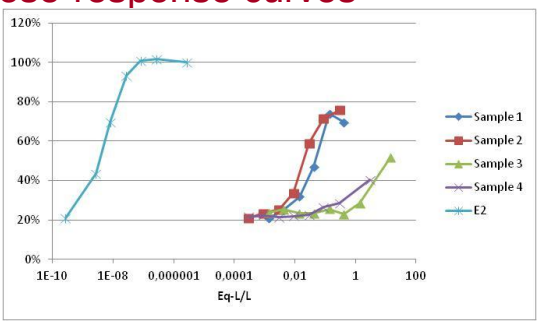
$$\text{Biol-TEQ} = ECx_{E2} / ECx_{sample}$$

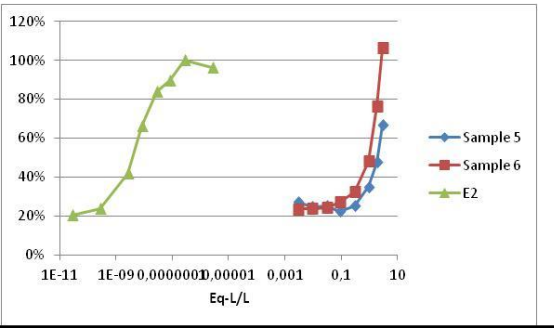
$$\text{Chem-TEQ} = \Sigma\, TEFi \,.Ci$$

with $TEFi = EC50_{E2} / EC50i$

Typical and non-typical dose-response curves







Assessment of complex mixtures using in vitro assays

Application to complex mixtures : theoretical approach

Mass balance has proven useful in many situations (e.g. Hormone-contaminated effluents) ...

BUT:

Variation between models :

- ☞*TEFs will vary across bioassays : check the sensitivity/specificity of your model !*

Practically, ideal cases are not so frequent. Bioanalysis of complex mixtures often lead to non typical curves : mixtures of full and partial agonists, ago/antago, cross-talk with other NR pathways,...

- ☞*How to deal with ? Cleo ?*