RIKILT
WAGENINGEN UR

In vitro and *in vivo* tools for NR research: from whole cell bioassays to –omics based methods

Toine F.H. Bovee*, Peter J.M. Hendriksen, Ron L.A.P. Hoogenboom, Ad A.C.M. Peijnenburg

Mammalian cell and yeast based bioassays

Bioassays based on mammalian cell lines are often very sensitive, showing maximal responses to steroids in the pM range. *In vivo*, however, 1 nM 17β-estradiol, the circulating level in premenopausal women, is required to maximally activate the ERα. The sensitivity of many mammalian cell lines is most likely reflecting the selection of these tumour cell lines during competition of growth. It's thus questionable whether these cell lines are the most suited models to study NR activity. Yeast based assays show comparable sensitivities towards natural hormones as normal healthy human cells and do not suffer from crosstalk of other NRs. Combined with models to mimic metabolism and bioavailability they offer great tools to specifically study NR activity.

ITX: a classic example of an endocrine disrupting chemical?

Responses of ITX in whole cell biosensors

ITX, 2-isopropylthioxanthone, is a photoinitiator widely used in printing ink. Figure 1 shows that ITX is able to activate the Ah-receptor in rat liver H4IIE cells and exerts both anti-estrogenic and anti-androgenic activity in yeast based bioassays, expressing green fluorescent protein (yEGFP) in response to estrogens and androgens respectively.

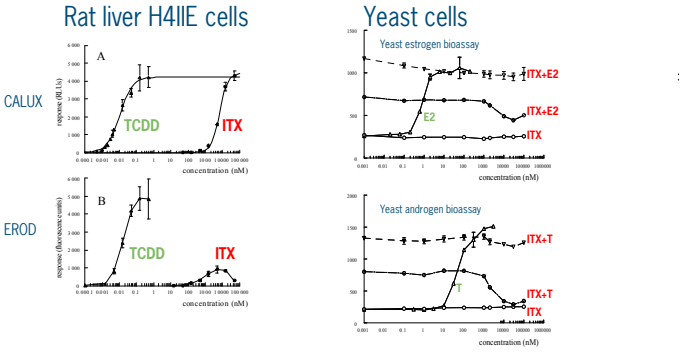


Figure 1. Responses of TCDD, E2, T and ITX in the DR-CALUX®, yeast estrogen and yeast androgen bioassays. In the latter two bioassays, ITX was co-administrated with doses' of estradiol (E2) and testosterone (T) that gave half maximal and near maximal responses (ITX + E2 and ITX + T).

In vitro gene expression profiles in H4IIE cells: ITX vs TCDD

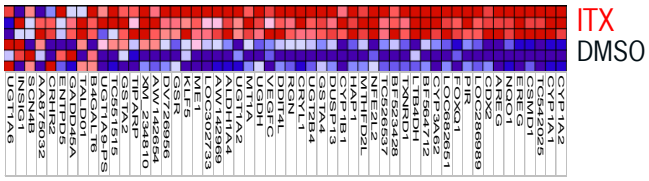
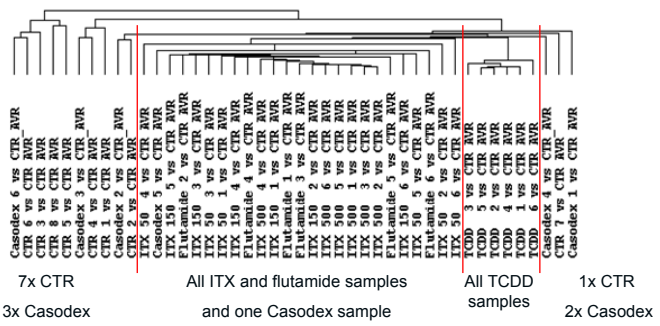


Figure 2 is the heat map that shows the relative expression levels of TCDD-affected genes in rat hepatoma H4IIE cells treated for 24 h either with 5 µM ITX or with the vehicle DMSO (control). Red: relatively high expression; blue: relatively low expression. (Genes differentially expressed by TCDD were selected on an average up-regulation in rat H4IIE hepatoma cells by ≥1.5-fold at 24 h in combination with a P-value of <0.01 (students t-test)).

The heat map shows that the vast majority of TCDD-up-regulated genes are also up-regulated by ITX.

In vivo micro array analysis on rat liver

Rats were treated orally with ITX and several control and reference compounds: DMSO (CTR), TCDD control for AhR agonist, Casodex for control of AR antagonist and Flutamide as a control for a compound displaying both AhR agonistic and AR antagonistic properties. Whole genome micro array analysis of the data and subsequent PCA clustering were performed. Resulting in the clustering as shown in figure 3. This confirms that ITX has an effect that resembles that of Flutamide: both the expected AhR activation and AR antagonistic effect. Although Casodex, the pure AR antagonist, has only little effect on gene expression in rat liver. (But Casodex showed clear AR antagonistic effects on organ weights, data not shown.)



RIKILT – Institute of Food Safety
P.O. Box 230, 6700 AE Wageningen
The Netherlands
Phone: +31 317 48 0391
Internet: www.rikilt.wur.nl
*Corresponding author: toine.bovee@wur.nl

Acknowledgments

The research described in this poster was funded by the Dutch Ministry of Agriculture, Nature and Food Quality