Different responses of zebrafish and human-based estrogenicity bioassays to selected environmental contaminants and their mixtures

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1. Introduction and objective

The activation of the estrogen receptor (ER) is a well described and conserved mechanism of action of endocrine disrupting chemicals in vertebrates. Effect-based tools (EBT) based on ER activation are widely used to monitor xeno-estrogens in environmental samples. We previously reported that established human- (MELN cells, expressing hERα) and zebrafish (ZELHα cells expressing zfERα, and ZELHβ2 cells, expressing zfERβ2) reporter cell lines are robust, reliable and sensitive to detect well-known ER ligands [1, 2]. However, they can respond to surface water and effluents samples in different ways, with samples being selectively active either on the human or on the zebrafish cell lines. Different responses may be due to non-identified active substances and/or to interactions with other chemicals. ER activation depends on the relative estrogenic potency of xeno-estrogens, which is specific to the ER subtype and the species of origin. In addition, specific factors to the cell context may influence the EBT response, such as metabolism (e.g. conversion into more potent/inactive metabolites) and potential interactions with other signaling pathways (e.g. AhR).

In this context, our study aims at comparing the response of zebrafish and human-based bioassays not only to selected environmental relevant pollutants, but also to their combination in order to simulate better the presence of complex mixtures in the field. Interactions between chemicals were taken into account by (1) assessing both activation and inhibition of ER response, (2) using concentration addition (CA) model to predict additive effects, and (3) combining active with non-active compounds. This work was performed within the EU FP7 project SOLUTIONS [3].

2. Material and methods

2.1 Human and zebrafish-based *in vitro* bioassays

Table 1: Description of the 3 reporter cell lines used is this study.

	MELN [4]	ZELHα [2]	ZELHß2 [2]
Species	Human	Zebrafish	Zebrafish
Target	hERα	zfERα	zfERß2
Cell context	Breast cancer (MCF-7)	Liver cells (ZFL)	Liver cells (ZFL)

2.2 Selected chemicals, single chemical testing and multi-component mixture experiments

From a recently published list of priority compounds for effect-based monitoring in European freshwaters [5] we selected 12 chemicals (fig. 1) for dose-response analysis. Each chemical was tested in triplicate/experiment in the range of 0.03 – 30 µM, alone and in presence of a fixed estradiol (E2) concentration and in every assay. Two 12-component mixtures were designed with fixed ratios of the compounds. Mixture 1 (M1) corresponds to high concentrated mixture, while mixture 2 (M2) was designed on the basis of an environmentally relevant mixture scenario. Predictions according to the concentration addition (CA) model were made for activation and inhibiting chemicals for each bioassay.

3. Results

3.1 (Anti)estrogenic activities of chemicals on human and zebrafish-based bioassays (fig. 1)

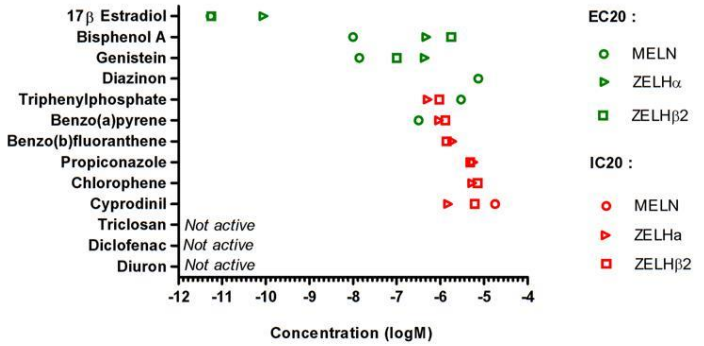

Figure 1: Single chemical evaluation. *ER activation (EC20, green) or inhibition (IC20, red) of the 12 chemicals in the 3 reporter cell lines. Each point represents the mean of min. 2 independent experiments.*

Biphenol A (BPA) and genistein were active on all bioassays but with different sensitivity. Significant differences between bioassays were noticed for some substances. Diazinon, triphenyl phosphate (TPP) and benzo(a)pyrene (BaP) induced ER transactivation only in the MELN cells. In contrast, TPP and BaP inhibited E2-induced reporter gene response in ZELHα and ZELHβ2 cells. Chlorophene, propiconazole, and benzo(b)fluoranthene (BbF) were inactive on MELN cells, but inhibited E2 response in ZELHα and ZELHβ2 cells.

3.2 Different responses of human and zebrafish-based bioassays to the 12-component mixtures (fig. 2)

In MELN cells, the estrogenic activity of mixture M1 was driven by genistein, BPA and TPP, and agreed well with the additivity assumption by CA. M1 had a slight inhibiting effect on E2 response that was, however, more potent than predicted. In ZELHα and ZELHβ2 cells, no estrogenic activity of M1 could be detected. M1 was predominantly inhibiting E2-induced response, which agreed well with the mixture predictions. This inhibition, driven by TPP and propiconazole, could explain the lack of estrogenic activity observed, in particular in ZELHβ2 cells. Further work is in progress to study interactions by grouping estrogenic and anti-estrogenic chemicals and testing M2.

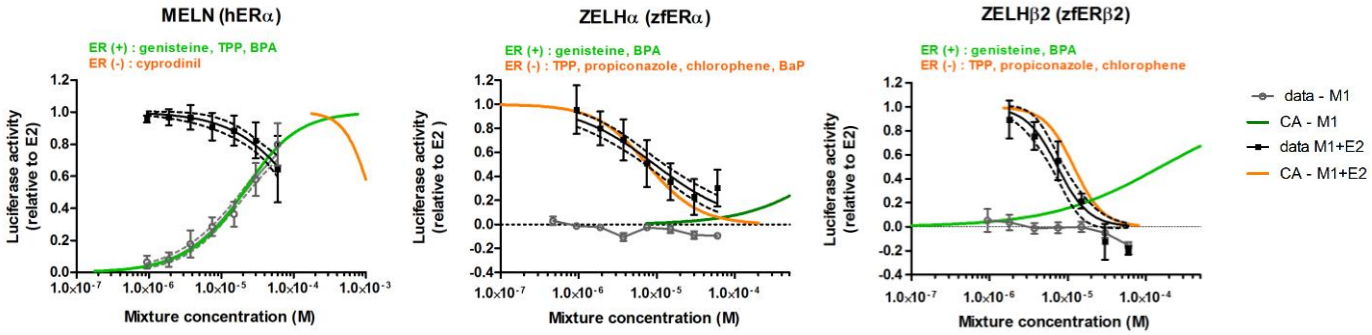

Figure 2: Response of MELN cells, ZELHα, and ZELHβ2 cells to M1 tested up to 1.2E-4 M. *Mean (circles) +/- SD of all replicats (n≥6). A two parametric nonlinear regression model was fitted (black line + 95% CI). Orange line: CA prediction for inhibiting chemicals indicated by ER(-); green line: CA prediction for activating chemicals indicated by ER(+). Chemical concentration at 1.2E-4 M of M1 (in M): BaP 3E-8, BbF 1E-7, BPA 7E-7, chlorophene 9E-6, cyprodinil 1E-6, diazinon 6E-9, diclofenac 3E-5, diuron 6E-7, genistein 1E-7, propiconazole 6E-5, TPP 1.5E-5, triclosan 3.5E-6.*

4. Conclusion and perspectives

The evaluation of single chemicals provided novel information on environmental pollutants of concern and highlighted marked differences for some chemicals that behave as (anti)estrogenic in some models but not in other (e.g.TPP, propiconazole). Interestingly, similar differences between models were observed when the chemicals were pooled into a mixture, demonstrating that both the cell lines (fish vs human) and the effects of antiestrogenic chemicals influence the estrogenic response. In line with results of environmental samples, it raises the question of cross-species differences and fish-specific effects that warrants further investigation in *in vivo* fish models. Altogether, the study shows the importance of the cell model for water quality biomonitoring. Future work will focus on the refinement of estrogenicity assessment by taking into account anti-estrogenic effects, and thus, providing novel insight into environmental contamination by EDC.

References

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