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# AUGMENTATION OF THE CYP INDUCTION TEST METHOD IN THE HUMAN HEPATIC HEPARG™ CELL LINE AND APPLICATION TO METABOLISM DISRUPTING COMPOUNDS

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## Context

Many human xenobiotic metabolizing enzymes (XMEs) are **inducible** by a number of **drugs** and **environmental chemicals**.

The induction of **Cytochrome P450 (CYP)** enzyme activities can have an impact on the fate as well as the biological effect(s) of xenobiotics.

In addition, since many CYPs are also involved in endogenous metabolic pathways of anabolism and catabolism, the **modulation of their activity** can also impact endogenous physiological functions, energy metabolism and signalling pathways (including hormones metabolism).

This type of mechanism is notably suspected for a number of endocrine disruptors, known as "metabolic disruptors".

The **standardization of *in vitro* strategies** for evaluating the induction of CYP enzyme functional activities constitutes a major challenge in toxicology.



## Objectives & Strategy

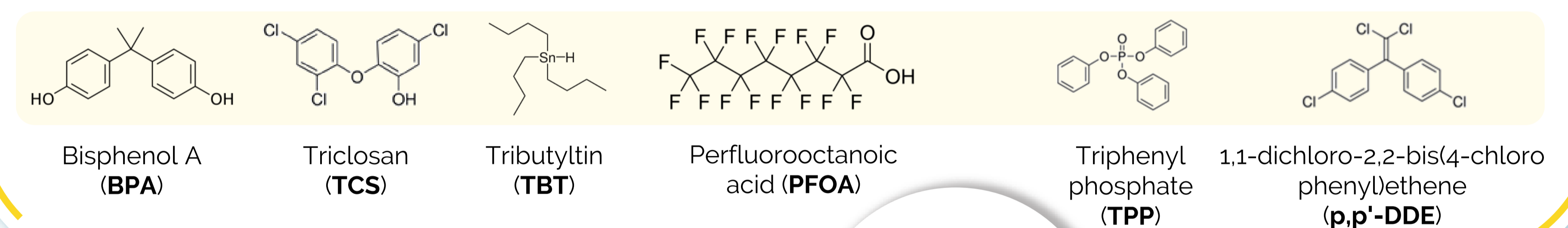
Within the GOLIATH project (EU Horizon 2020, grant n°825489), investigations are conducted to examine the potential of model **Metabolism Disrupting Compounds (MDCs)** to exert their effects through the modulation of functional XME activities.

The **central objective** of this study is to **contribute to the development and validation of biochemical and analytical *in vitro* approaches allowing a better assessment of the functional induction of XME enzymes**, in order to better characterize the effects related to exposure to chemical contaminants.

Phase I activities are notably assessed with the aim to examine the potential of model MDCs to induce **key CYP activities**, that are **inducible in humans**:

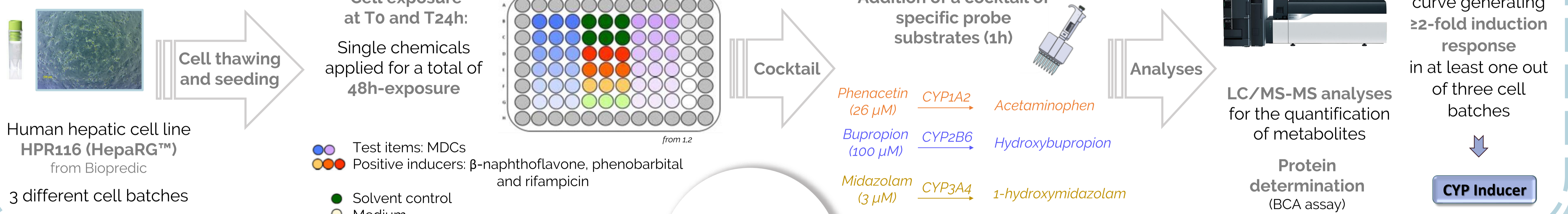
The **CYP induction test method**, first developed and validated with ten pharmaceutical proficiency chemicals [TM2009-14 (EU)]<sup>1,2</sup>, was optimized and applied to **6 model MDCs** at 6 concentrations, including both persistent and non-persistent chemicals:

**CYP1A2, CYP2B6, CYP3A4**



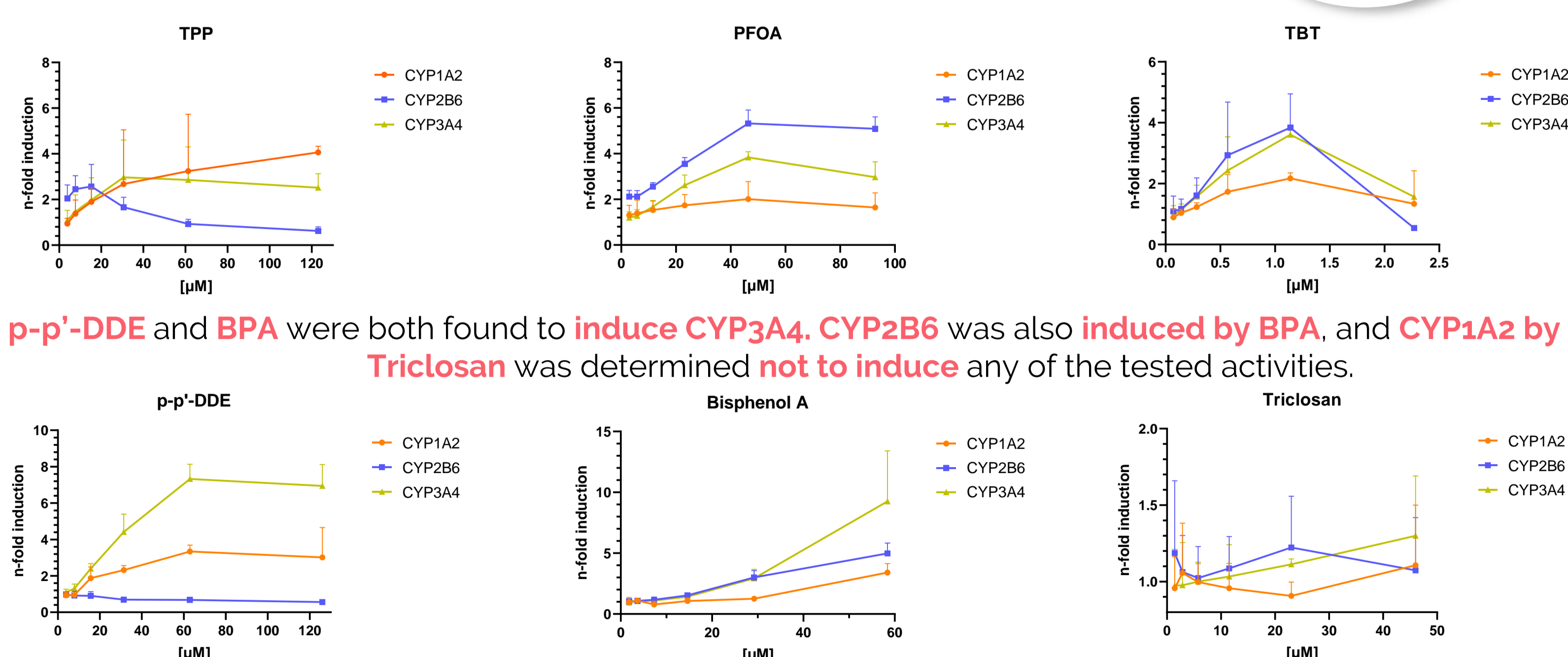
## Protocol

- **Solubility and cytotoxicity assays** were performed (CellTiter Blue® method) to select the highest non cytotoxic concentration
- **CYP test method protocol:**



## Results

TPP, PFOA and TBT induced the 3 CYP activities assessed.



p,p'-DDE and BPA were both found to **induce CYP3A4**. CYP2B6 was also **induced by BPA**, and CYP1A2 by p,p'-DDE. Triclosan was determined **not to induce** any of the tested activities.

Key results are summarised in these tables:

	Induction				Induction		
	CYP1A2	CYP2B6	CYP3A4		CYP1A2	CYP2B6	CYP3A4
TPP	Yes	Yes	Yes	TPP	+++	++	+++
PFOA	Yes	Yes	Yes	PFOA	+	+++	++
TBT	Yes	Yes	Yes	TBT	+	+	++
p,p'-DDE	Yes	No	Yes	p,p'-DDE	++	N	+++
BPA	No	Yes	Yes	BPA	~	++	++
TCS	No	No	No	TCS	N	N	N

N = not an inducer  
 ~ = uncertain inducer  
 + = weak inducer, 2 consecutive concentrations generating a response ≥ 2 to < 3-fold induction in at least one batch  
 ++ = moderate inducer, 2 consecutive concentrations generating a response > 3 to < 4.5-fold induction in at least one batch  
 +++ = strong inducer, 2 consecutive concentrations generating a response > 4.5-fold induction in at least one batch

## Conclusions

The test method was developed and optimized to investigate the potential of chemicals to induce the activity of key CYP enzymes (CYP1A2, CYP2B6 and CYP3A4), mediated via AhR, PXR and/or CAR receptors in the human hepatic cell line HPR116.

This test method aims at identifying chemicals that, through CYP induction, may impact the biotransformation of endogenous as well as exogenous substances.

We found that **5 out of the 6 MDCs tested were determined to induce at least one of the 3 specific CYP activity assayed**.

Results obtained for the set of model MDCs provide valuable information about the **possible involvement of CYP induction mechanisms** that are triggered by some of these compounds and **may contribute to their Mode of Action (MoA)**.

## References:

- <sup>1</sup> Cytochrome P450 (CYP) enzyme induction *in vitro* method using cryopreserved differentiated human HepaRG™ cells. EURL ECVAM - European Union Protocol(s)/SOP(s) n°194
- <sup>2</sup> Bernasconi C, et al. Validation of *in vitro* methods for human cytochrome P450 enzyme induction: Outcome of a multi-laboratory study. Toxicol In Vitro. 2019 Oct;60:212-228.