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Modulation of phase I and phase II xenobiotic metabolism enzymes (XMEs) induced by environmental and food contaminants

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Introduction

The expression of several human xenobiotic metabolizing enzymes (XMEs) can be modulated (induction, repression) by a number of drugs and environmental chemicals. XMEs induction is by far the most common adaptative process for biological systems exposed to xenobiotics, compared to repression. Activities modulation more often concerns Phase I (CYP450) reactions, although phase II enzymes (UDP-glucuronosyltransferases, sulfotransferases...) may also be impacted, being partly regulated through similar pathways, including through the activation of key Nuclear Receptors such as CAR and PXR, which act as 'sensors' of exogenous substances exposure. Any modulation of XMEs can potentially impact the biological effect(s) of a given xenobiotic, its metabolic fate, but also endogenous physiological functions and signalling pathways. Indeed, lasting modulation of the functionality of XMEs may deeply impact the metabolic fate of exogenous chemicals, and general metabolism, since these key enzymes, in addition to biotransformation of xenobiotics and detoxification pathways, are involved in numerous biochemical reactions, including the anabolism and catabolism of sugars, lipids and hormones.

Objectives & Strategy

Metabolism is the primary defense against xenobiotics, and **Xenobiotic Metabolizing Enzymes (XMEs)** play an essential role in the protection of organisms

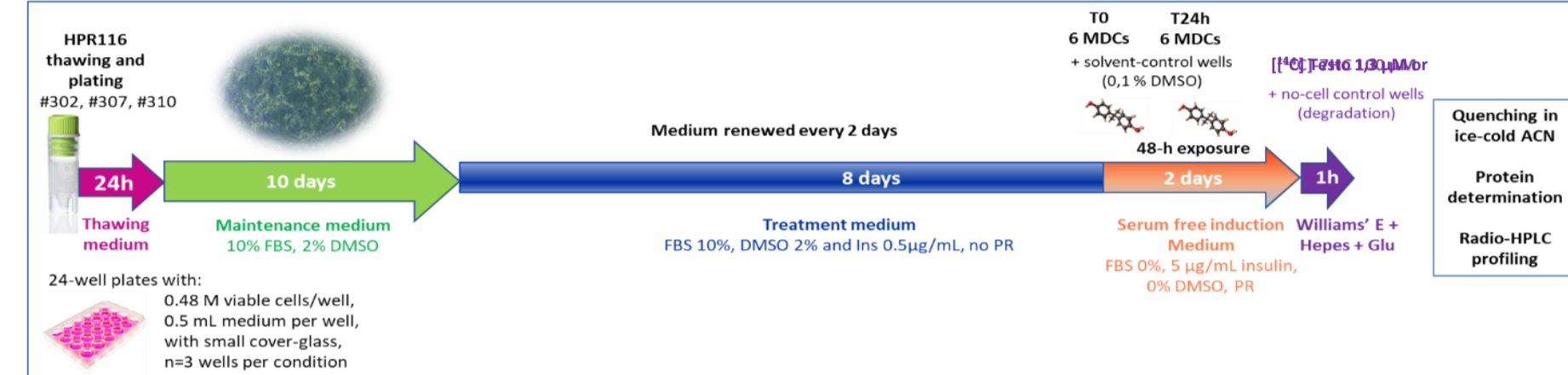
⇒ We investigated the potential of Metabolic Disruptor Chemicals (MDC) to modulate the metabolic capabilities of human hepatocytes (HPR116 cells), using labelled reference molecules for phase I (testosterone) and phase II (7-hydroxycoumarin), respectively.

HPR116 cells were exposed to a non-cytotoxic concentration range of each of Goliath's model MDCs (BPA, PFOA, TBT, pp'DDE, triclosan and triphenylphosphate) for 48 h or 10 days, following a 10-day recovery period after thawing. Then, we examined the modulation of phase I and phase II metabolism by carrying out, in parallel, incubations with testosterone and 7-hydroxycoumarin, respectively, as probe substrates.

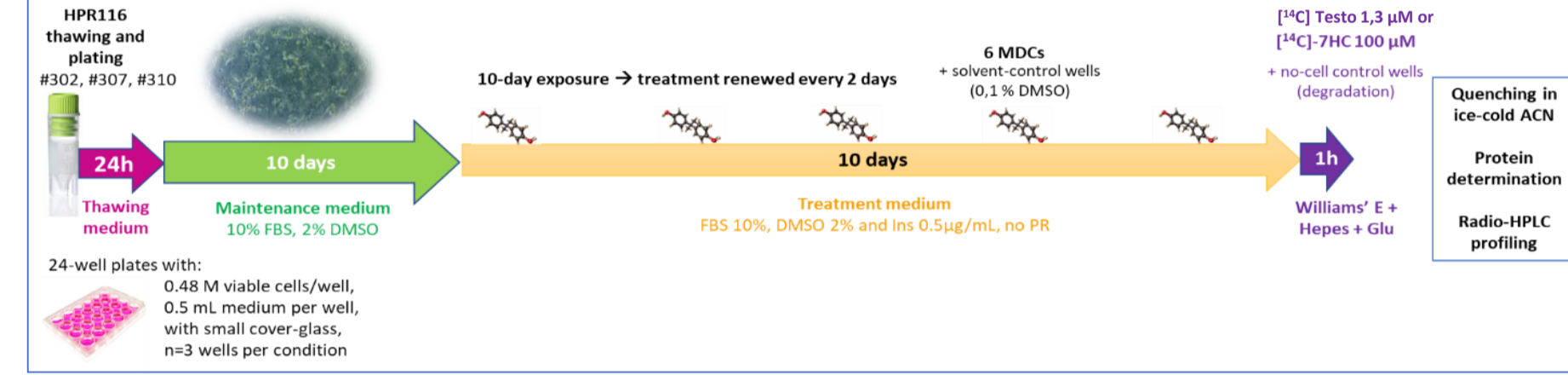
Experimental design

Cell experiments

General principle of the protocol used for the 48h scenario

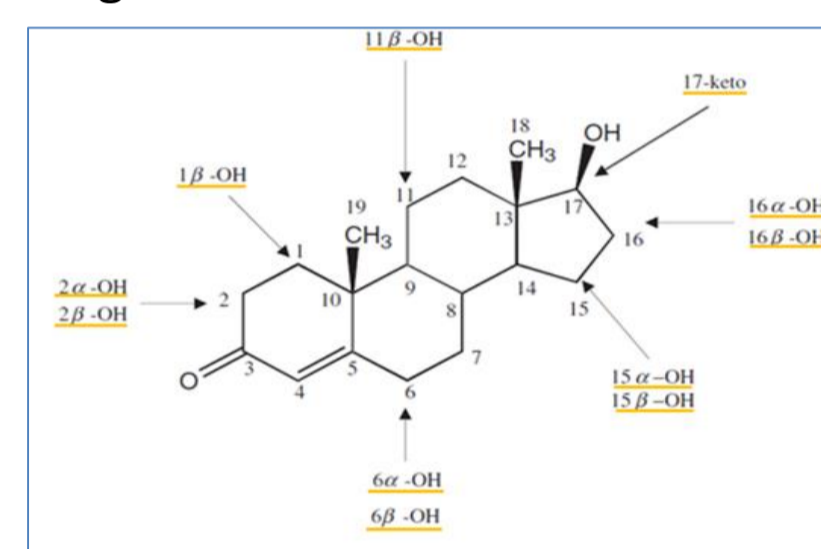


General principle of the protocol used for the 10d scenario



Phase I activities

Testosterone assay. Testosterone is an endogenous molecule, which oxidation products (metabolites) by various CYP activities have been demonstrated to be regioselective.



Each hydroxylation position of Testosterone is carried out by one (or several) CYP isoforms. Further developments of this approach have been carried out using [¹⁴C]-Testosterone. Following the incubation testosterone with the cells, incubation media were analyzed using radio-HPLC to profile all Testosterone metabolites.

Fig 1: Regio-selective hydroxylation sites of Testosterone by various CYP activities. (Adapted from Waxman et al. 1991)

The different radioactive peaks were quantified to allow a statistical comparison in exposed vs control conditions.

Phase II activities

7-hydroxycoumarin assay. We selected for our studies the reference substance 7-hydroxycoumarin, relying on a radio-labelled substrate, to allow a full and precise profiling and quantification of metabolites based on the analysis of HPR116 cell incubation media. [¹⁴C]-7-hydroxycoumarin ([¹⁴C]-7HC) was used exactly with the same protocol of exposure (48 h as well as 10 d), to explore the functionality of phase II XMEs.

Incubation media were analyzed using radio-HPLC. The different radioactive peaks were quantified to allow a statistical comparison in exposed vs control conditions.

It is possible to assess the **global phase II capacities (sulfotransferases and UDP-glucuronosyltransferases)** of a given *in vitro* system following xenobiotic exposure.

Results

Phase I activities modulated by the 6 Goliath's MDCs

⇒ TPP and TBT had no effect on any of the HPR116 cell activities involved in testosterone metabolism. Conversely, BPA and PFOA clearly induced the 16 α -hydroxylase activity in the single exposure protocol, and also had slighter effects on other activities. TCS exerted a limited but significant effect on several activities, mainly in the repeated exposure scenario. The most potent compound was p,p'-DDE, which impacted numerous activities, either with an induction or a repression effect. Most of the activities related to quantitatively major testosterone metabolites were found to be impacted by p,p'-DDE exposure.

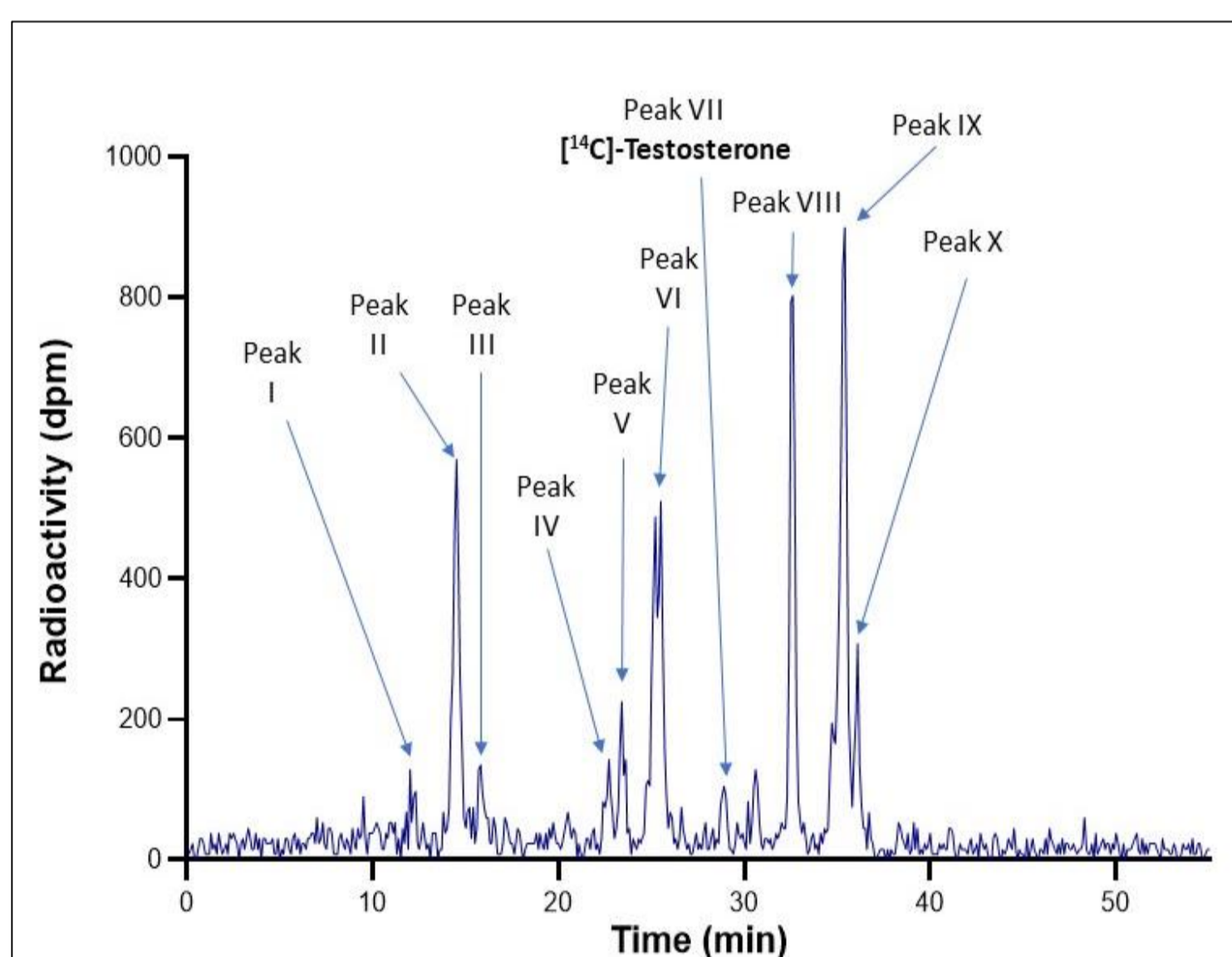


Fig 2: Typical radio-chromatogram of a medium sample of HPR116 cells exposed to 1.3 µM [¹⁴C]-Testosterone for 4h (HPR116 lot #310), here following a 48h exposure to DDE.

⇒ Less activities were found to be impacted in HPR116 cells by the exposure to other Goliath's MDCs. Further investigations (including LC-MS confirmation of key testosterone metabolites) are being carried out as well for these substances.

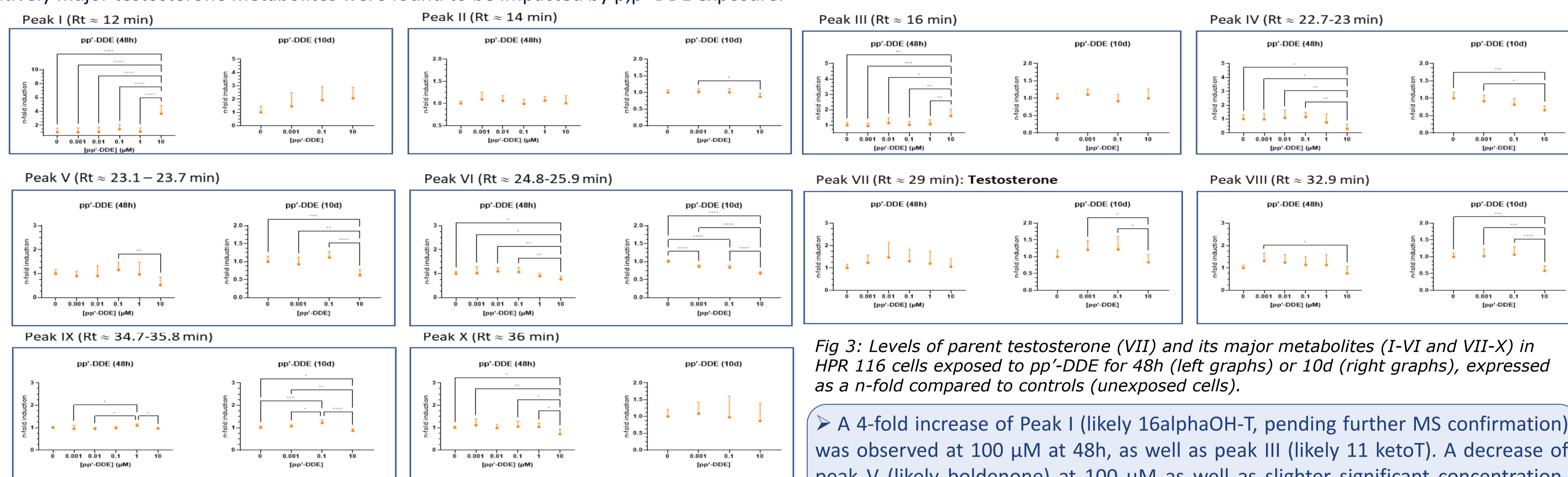


Fig 3: Levels of parent testosterone (VII) and its major metabolites (I-VI and VII-X) in HPR 116 cells exposed to pp'-DDE for 48h (left graphs) or 10d (right graphs), expressed as a n-fold compared to controls (unexposed cells).

⇒ A 4-fold increase of Peak I (likely 16 α OH-T, pending further MS confirmation) was observed at 100 µM at 48h, as well as peak III (likely 11 ketoT). A decrease of peak V (likely boldenone) at 100 µM as well as slighter significant concentration-dependent decreases of peaks IV, VI and X, and of peak VIII were also observed.

Phase II activities modulated by the 6 Goliath's MDCs

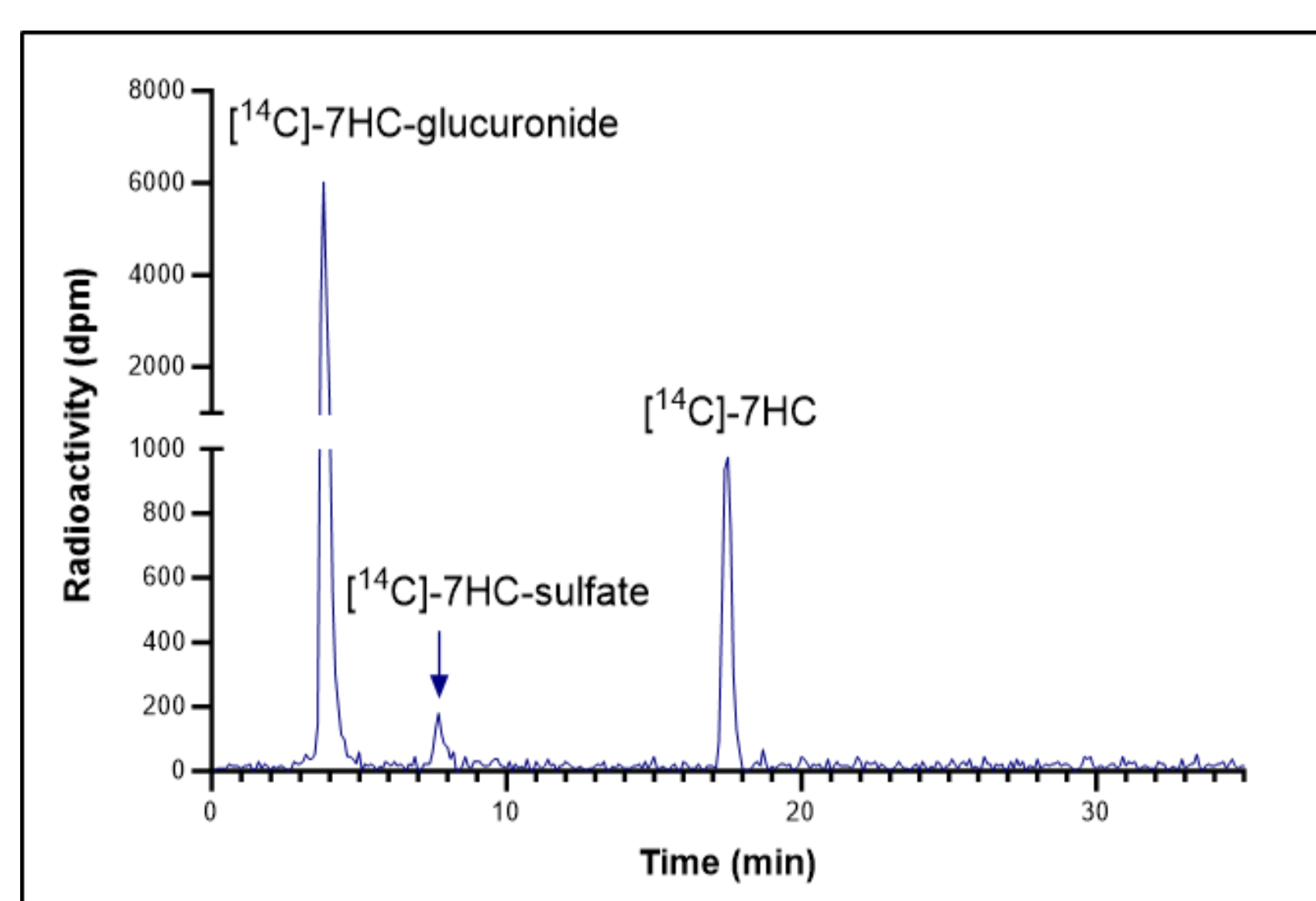


Fig 4: Typical radio-chromatogram of a medium sample of HPR116 cells exposed to 10 µM [¹⁴C]-7HC for 1h (HPR116 lot #302), here following a 48h exposure to TCS.

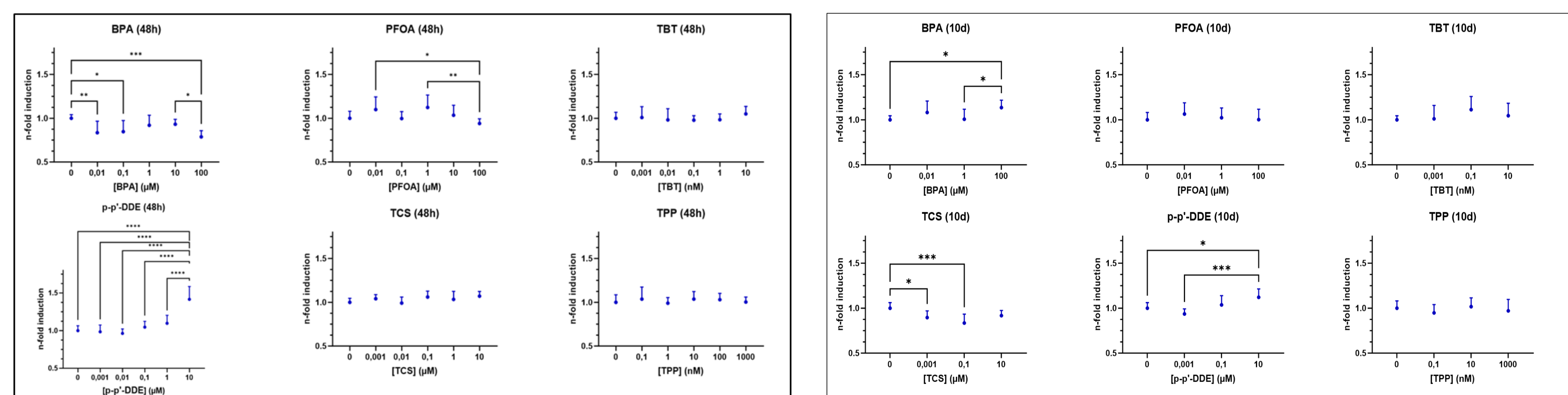


Fig 5: Effect of GOLIATH MDCs on the functionality of UDPGT, explored using labelled 7-HC. 7-HC glucuronide formation is expressed as n-fold (compared to controls) following a 48h (left panel) and 10 d (right panel) of exposure to the 6 substances.

For glucuronidation activities, significant changes were observed for the highest concentration of p,p'-DDE (10 µM). In addition, significant differences were observed for PFOA and BPA, with, for the latter, a possible non-monotonic dose-response. The 10 d scenario with repeated exposure to MDCs confirmed an effect of p,p'-DDE exposure for the highest concentration tested (10 µM). BPA was found to have a slight but significant increase effect on glucuronidation activities. Conversely, TCS, at low concentrations, significantly decreased these activities.

Conclusions

An extensive metabolism of Testosterone was observed in HPR116 cells, which phase I oxidations are known to be regio-selective. These experiments with labeled testosterone will be followed by further structural identifications relying on mass spectrometry. These will ultimately allow to deliver key information about the isoforms of CYPs involved in the specific hydroxylation's modulation highlighted in our experiments. A notable induction of glucuronidation activities (50% increase) was clearly demonstrated for p,p'-DDE, and a significant repression of this activity occurred for BPA. Sulfation pathways were not impacted by MDC exposure. However, experiments carried out for an extensive set of conditions and concentrations clearly suggest that though sulfation activities are expressed in HPR116 cells, they are limited compared to glucuronidation. These results, once the MS identification/confirmation steps are completed for testosterone, will provide a very solid ground for the further exploration of modulatory effects of MDCs on phase I and II XMEs activities.