



Funded by the Seventh Framework
Programme of the European Union



Project full title:
Hepatic and Cardiac Toxicity Systems modelling

Project acronym:
HeCaTos

Collaborative project
HEALTH.2013.1.3.-1:
Modelling toxic response in case studies for predictive human safety assessment

FP7-HEALTH-2013-INNOVATION-1-602156-HeCaTos

**Deliverable Report D4.3:
Report on model-based dose response correlations**

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Work package 4

Due date of deliverable: M40
Actual submission date: M41

Start date of project: October, 2013

Duration: 60 months

Maastricht University (UM)

Project co-funded by the European Commission within the 7th Framework Programme (2013-2018)		
Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Contributions to deliverable - Internal review procedure

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PUBLISHABLE SUMMARY

We have developed an integrative multiscale approach for the establishment of dose response correlations which describes cellular responses within a whole-body context. The approach, termed PICD (PBPK-based *in vivo* contextualization of *in vitro* toxicity data) integrates *in vitro* toxicity data at the cellular scale into physiologically-based pharmacokinetic (PBPK) models at the organism level. Both scales are coupled through putting the *in vivo* drug concentration-time profiles in the extracellular environment of the target organ to the level of the *in vitro* drug assay.

PICD was exemplary applied in three published studies: (1) a clinical case study of acute azathioprine-induced liver injury (Thiel et al., 2016), (2) a comparative analysis of drug-induced hepatotoxicity in 15 hepatotoxicants (Thiel et al., 2017a) and (3) an analysis of the stimulatory and inhibitory effects of caffeine on paracetamol toxicity (Thiel et al., 2017b).

OBJECTIVES

1. Develop a concept for vertical model integration of molecular networks into whole-body PBPK models;
2. Establish model-based dose-response correlation in PBPK-based multi-scale models;
3. Develop a predictive modelling platform for drug-induced liver and heart injury.

INTRODUCTION

Physiologically-based pharmacokinetic (PBPK) modelling supports a mechanistic representation of absorption, distribution, metabolism and elimination (ADME) processes governing drug pharmacokinetics (PK) within the human body (Kuepfer et al., 2016). In particular, PBPK models are based on a large amount prior physiological information which enables a quantitative description of mass transfer within the body of an organism. In addition to the physiological and anthropometric information, PBPK model development requires information regarding the physicochemistry of the drug whose ADME behaviour is to be simulated. This compound-specific information (such as lipophilicity, molecular weight or fraction unbound) is routinely available for many marketed drugs and can be used to parametrize so-called distribution models to describe organ-plasma partitioning. This allows in turn to predict and to simulate tissue-specific concentration profiles in different organs and tissues throughout the body which would otherwise be not accessible within an *in vivo* context. We here used this particular property of PBPK modelling to simulate *in vitro* toxicity data within a whole-body *in vivo* context (PICD, Figure 1, Thiel et al., 2016). *In vitro* toxicity data for different hepatotoxicants were integrated into drug-specific whole-body PBPK models to translate drug-induced *in vitro* findings to an actual *in vivo* situation. Thus drug-specific dose-response correlations at the whole-body level could be thus described. For our analyses we used *in vitro* toxicity data from the Open TG-GATEs library (Igarashi et al. 2015) at the cellular scale which were correlated with corresponding PBPK-simulated concentration-time profiles at the organism level. PICD thus supports the model-based translation of preclinical *in vitro* toxicity data into an *in vivo* context through model-based dose-response correlations.

RESULTS

The preparatory step for PICD is the establishment of drug-specific PBPK models. Thus, *in vivo* doses can be identified that lead to drug exposure at the target organ which is equal to the *in vitro* concentration in the laboratory assay (Figure 1). In an iterative search, *in vivo* drug doses are next identified such that *in vitro* drug exposure in the assay equals the interstitial area under the curve in the liver at each experimental time point (Figure 1). Thus, cellular response curves obtained from *in vitro* toxicity may be correlated through *in vivo* drug dosed administered in human patients. The thus established drug-response correlations may then be used to predict *in vivo* drug responses in relevant gene ontology (GO) terms (Ashburner et al. 2000), as well as in human pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000) and in toxicity-related pathways (TOX) (SABiosciences).

Within HeCaToS, PICD has been exemplarily applied to three prototypical use cases:

1. A clinical case study for acute azathioprine overdoses (Thiel et al., 2016). PBPK models for the hepatotoxicant azathioprine were developed and carefully validated against literature PK data (Figure 2). Concentration–time profiles were then simulated in the interstitial space of the liver and correlated to *in vitro* transcriptomics data from the Open TG-GATEs library (Igarashi et al. 2015). Thereby, correspondent cellular drug response profiles were simulated following administration of a specific dose at whole-body level (Figure 3). The dose-response correlations were used to analyse reported clinical cases of acute azathioprine overdoses (Gregoriano et al. 2014).
2. A comparative analysis of drug-induced hepatotoxicity in 15 hepatotoxicants (Thiel et al., 2017a). PBPK models for 15 hepatotoxicants (APAP; amiodarone, AD; azathioprine, AZA; cyclophosphamide, CPA; cyclosporine A, CSA; diclofenac, DFN; erythromycin, ERY; erythromycin ethylsuccinate, ERY-PED; flutamide, FT; haloperidol, HPL; isoniazid, INH; phenobarbital, PB; phenytoin, PHE; rifampicin, RIF; simvastatin, SST; valproic acid, VPA) were developed and carefully validated against published literature PK data (Figure 4). Again, simulated concentration profiles in the liver interstitium were correlated to transcriptomics data from the Open TG-GATEs library (Igarashi et al. 2015). The dose-response profiles were used to describe the changes during the transition from therapeutic to toxic drug doses, respectively, henceforth referred to as *toxic change*. These toxic changes were analyzed amongst others at the functional level to quantitatively describe to what extent single or subset of drugs perturbed different functional classes of genes, such as kinases or metabolic enzymes, associated to key cellular processes (Figure 5). Also, genetic biomarker for specific cellular responses were identified and used to predict cases of drug-drug interactions (Thiel et al., 2017a).
3. An analysis of the stimulatory and inhibitory effects of caffeine on paracetamol hepatotoxicity (Thiel et al., 2017b). Additive effects during co-administration of caffeine (CAF) and acetaminophen (APAP) were also analysed with PICD. PBPK models were first developed for both drugs and validated (Figure 6). The dose dependent changes in the PD effect of APAP due to the co-administration of CAF were then calculated by using PICD (Figure 7-9). Overall, the co-administration of CAF led to a statistically significant perturbation ($p < 0.01$, two-sample t-test) of all considered key cellular processes (Figure 7). For example, analyzing PD responses at 8 h following single administration of APAP revealed a substantial impact on cell cycle G1/S and G2/M checkpoint regulation as well as on liver necrosis (Figure 7). When analyzing the impact of single administration of APAP and co-administration together with CAF on individual genes, the analyzed genes were additionally subdivided into their corresponding functional classes to allow a functional interpretation (Figure 8). In this gene-level analysis, PD responses of significantly perturbed genes induced by a single toxic dose of APAP and the corresponding PD effects provoked by a co-administration of CAF were

analyzed. Besides the identification of genes crucially affected by APAP, inhibitory and stimulatory effects of CAF on APAP were thereby investigated (Figure 8). In this context, a positive and negative PD effect value means that CAF increases or reduces the PD responses of APAP at the cellular level. Finally, a dose escalation study was performed to investigate cellular perturbations induced by single administration of APAP or co-administration with CAF for single genes (Figure 9)

To summarize, PBPK-based *in vivo* contextualization of *in vitro* toxicity data represents a generic approach for the establishment of dose-response correlations (Thiel et al., 2016). The applicability of the approach was illustrated for various clinical use cases (Thiel et al., 2017a, Thiel et al., 2017b). It should be noted that even though all cases discussed in this report refer to hepatotoxicity, cardiotoxicity could also be covered analogously given the availability of adequate cellular toxicity data. PICD hence represents a generally applicable approach to quantitatively translate *in vitro* results to *in vivo* situations. It is an often unresolved question whether or to which extent experimental design in the lab actually corresponds to clinical practice in living patients. PICD allows the predictions of cellular events in response to drug doses administered in human patients. It hence simulates events and causal chains which would otherwise not be accessible in living organisms. Overall, PICD might hence facilitate the understanding of drug-induced liver injury at patient level and thus may improve patient safety in clinical practice.

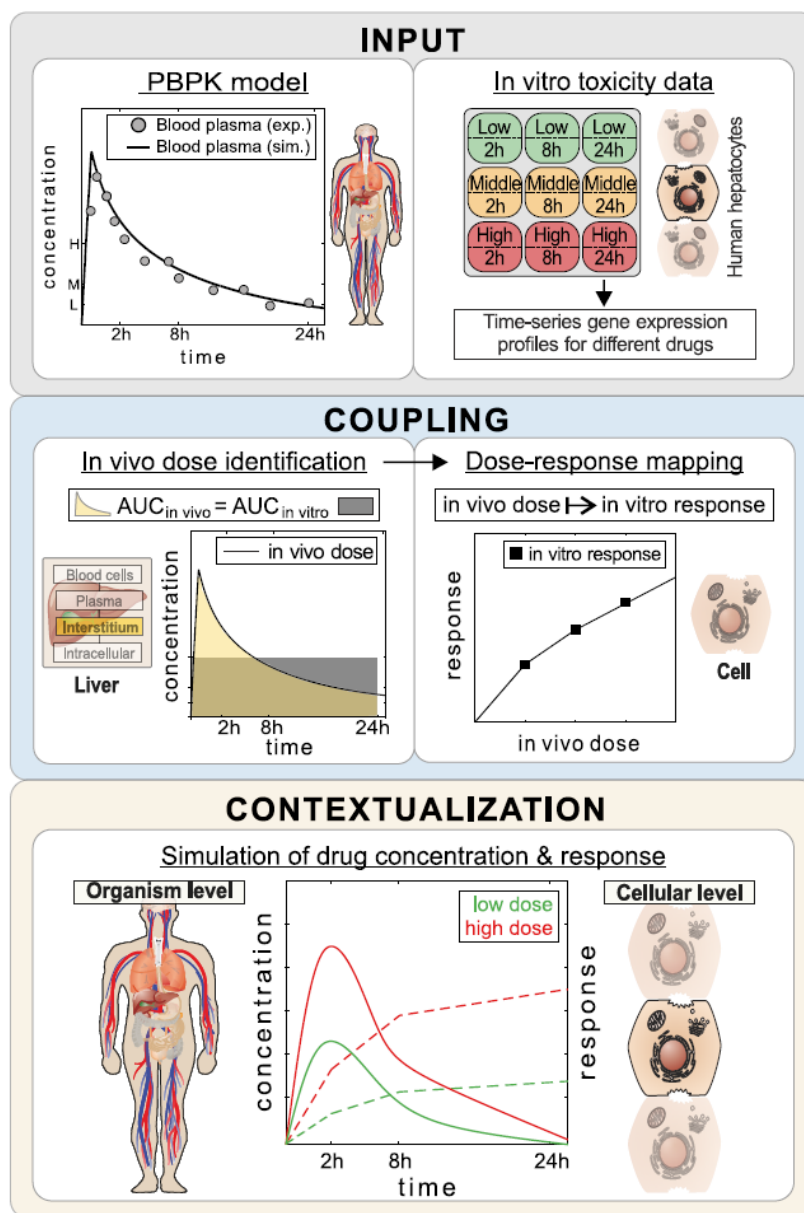


Figure 1: PBPK-based *in vivo* contextualization of *in vitro* toxicity data. **INPUT:** At the organism level, PBPK models are developed for specific drugs. At the cellular level, *in vitro* response data of compound-treated primary hepatocytes are analyzed. **COUPLING:** *In vivo* doses are identified, which are directly related to *in vitro* drug exposure ($AUC_{in\ vivo} = AUC_{in\ vitro}$). Time-dependent dose-response curves are built by mapping *in vivo* doses to *in vitro* responses. **CONTEXTUALIZATION:** By use of the time-dependent dose-response curves drug responses over time.

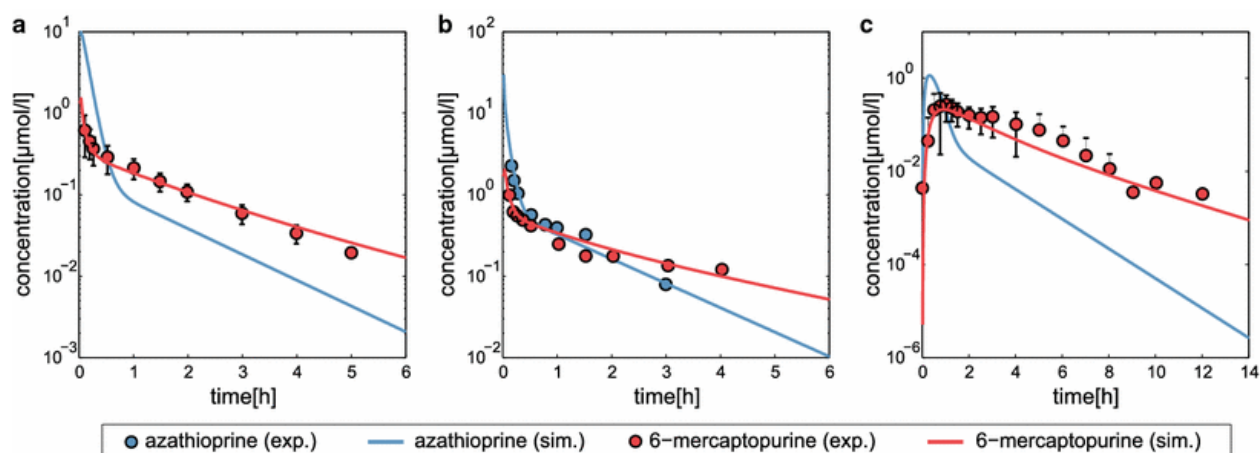


Figure 2: PBPK model development for azathioprine and validation. Simulated concentration-time curves (lines) for azathioprine (blue) and 6-mercaptopurine (red) were assessed with experimental PK profiles (circles) (Van Os et al. 1996). The reference PBPK model was then validated by evaluating simulated PK profiles with experimental PK data from different clinical studies (Odlind et al. 1986; Zins et al. 1997) (Table S2) not used to establish the reference model. Azathioprine was either administered intravenously (IV) or orally (PO). **a** Reference, 50 mg IV. **b** Validation, 100 mg IV. **c** Validation, 100 mg PO.

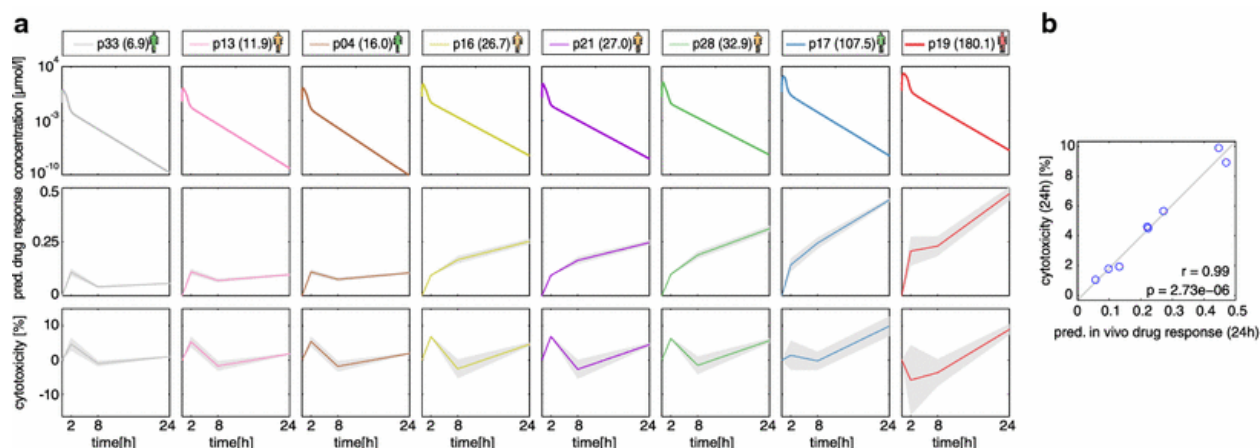


Figure 3: PICD applied on eight clinical cases of acute azathioprine overdose. **a** Simulated drug concentration-time profiles, corresponding predicted *in vivo* drug response of a critical toxicity-related pathway (DNA damage and repair), as well as predicted cytotoxicity for eight clinical cases following oral administration of different azathioprine overdoses. *In vivo* drug responses and cytotoxicity were predicted for both replicates to represent the variability (gray area) (Igarashi et al. 2015). The mean drug responses are shown as solid lines. Colors of patients indicate the highest Poisoning Severity Score (PSS) (Persson et al. 1998) of the occurred symptoms [none (green) = 0, minor (yellow) = 1, moderate (red) = 2]. The overdoses (mg/kg) are shown in brackets. **b** Correlation results of predicted *in vivo* drug response of DNA damage and repair at 24 h with predicted cytotoxicity values. Correlation analysis was performed by calculating Pearson's correlation coefficient r and the corresponding p value.

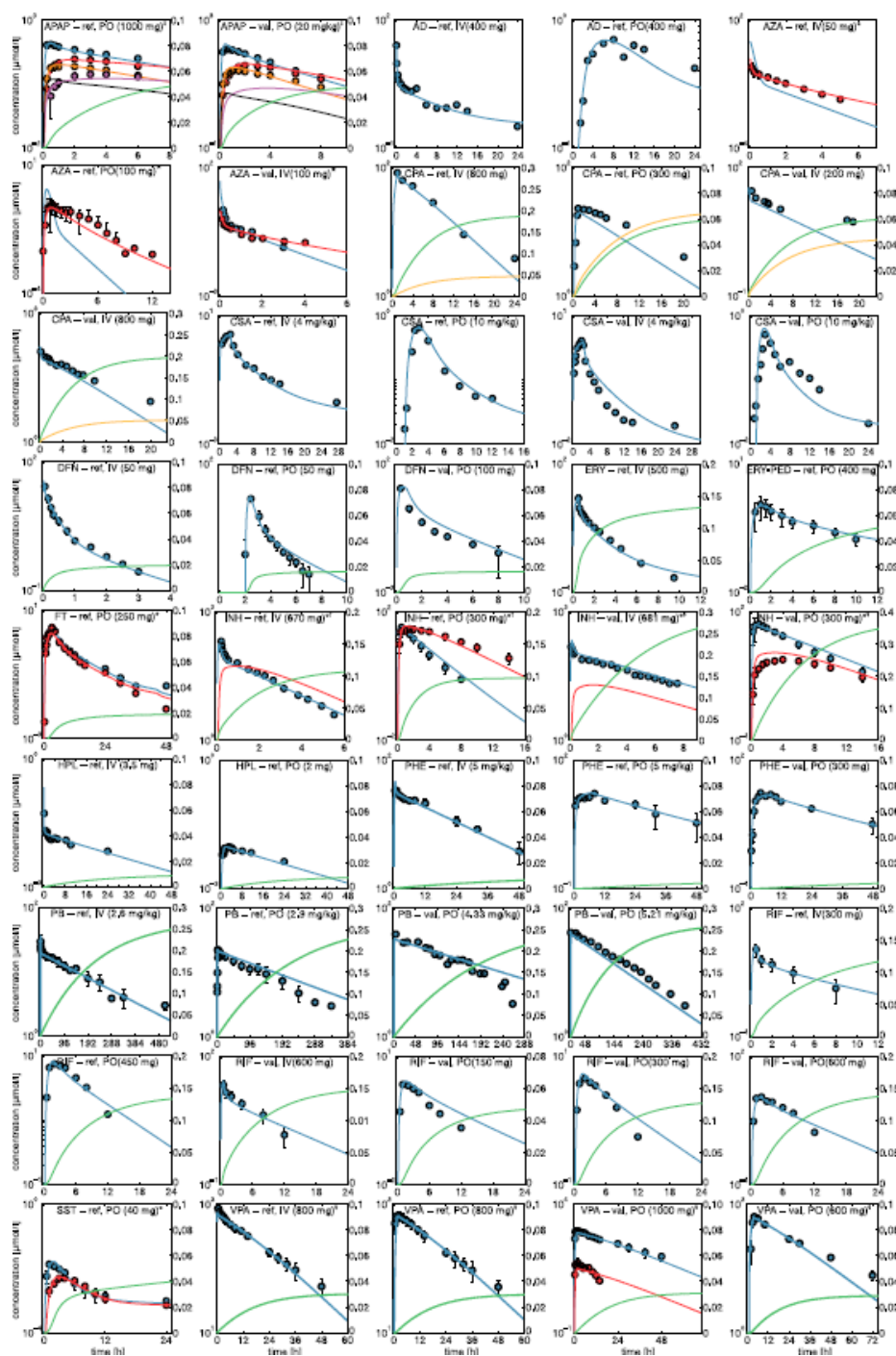


Figure 4: Human PBPK models of 15 hepatotoxicants. Simulated concentration-time curves (lines) for all parent drugs (blue) were assessed with experimental PK profiles (circles) used for developing reference (ref.) or validated (val.) human PBPK models. Drugs were either administered orally (PO) or intravenously (IV). Additionally, renal (green) and biliary (dark yellow) excretion rates were simulated. * Primary metabolites (red) 6-MP, 2-hydroxy-FT, acetyl-INH, and SST-acid; † APAP-glucuronide (red), APAP-sulfate (orange), APAP-cysteine (purple), and NAPQI (black); ‡ Rapid metabolizer; § Slow metabolizer; § Unbound plasma concentrations (red) (APAP; amiodarone, AD; azathioprine, AZA; cyclophosphamide, CPA; cyclosporine A, CSA; diclofenac, DFN; erythromycin, ERY; erythromycin ethylsuccinate, ERY-PED; flutamide, FT; haloperidol, HPL; isoniazid, INH; phenobarbital, PB; phenytoin, PHE; rifampicin, RIF; simvastatin, SST; valproic acid, VPA).

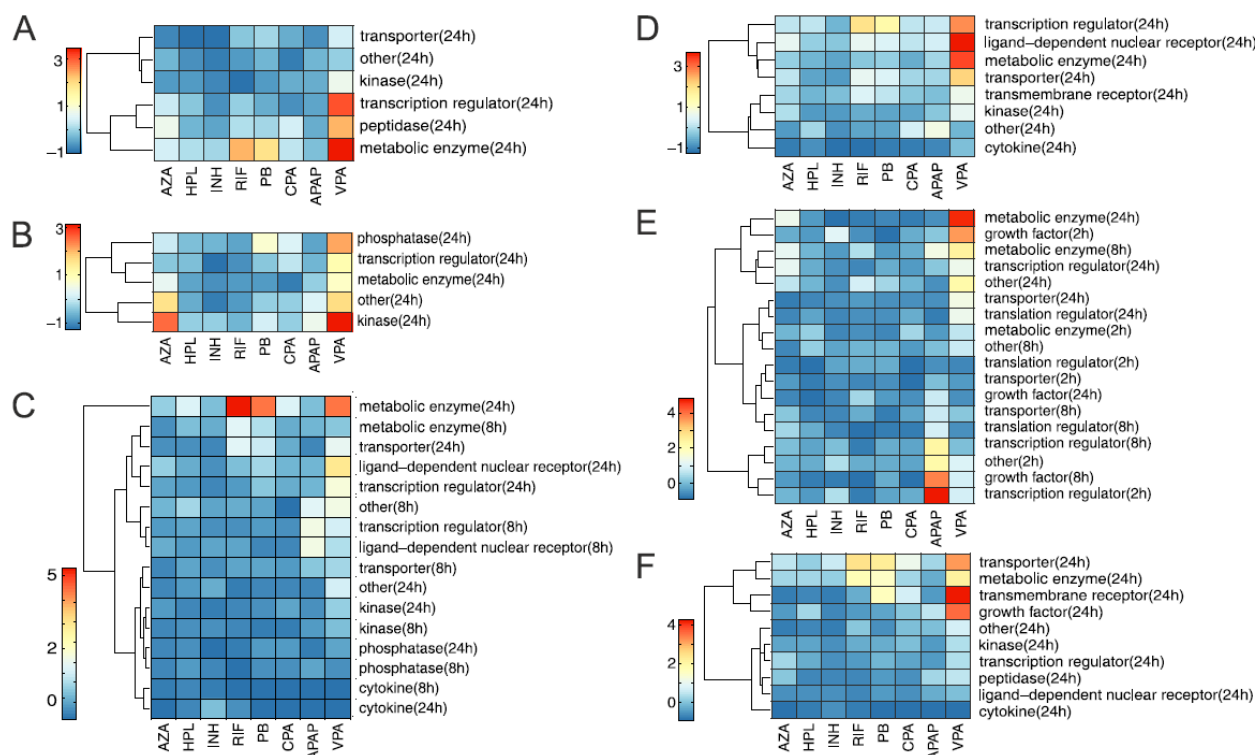


Figure 5: Toxic changes predicted for functional classes of genes involved in key cellular processes. The toxic changes were predicted for different functional classes of genes involved in the respective key cellular processes. All drugs belonging to the high-responsive group were considered. The color scale depicts toxic changes that were normalized over each heatmap. Normalization for each key cellular process is performed by subtracting the mean and by dividing the respective standard deviation.

- A. NRF-2 mediated oxidative stress response;
- B. 'Cell cycle G2/M DNA damage checkpoint regulation';
- C. 'PXR/RXR activation';
- D. 'LPS/IL-1 mediated inhibition of RXR function';
- E. 'Primary glomerulonephritis biomarker panel';
- F. 'Aryl hydrocarbon receptor signalling'.

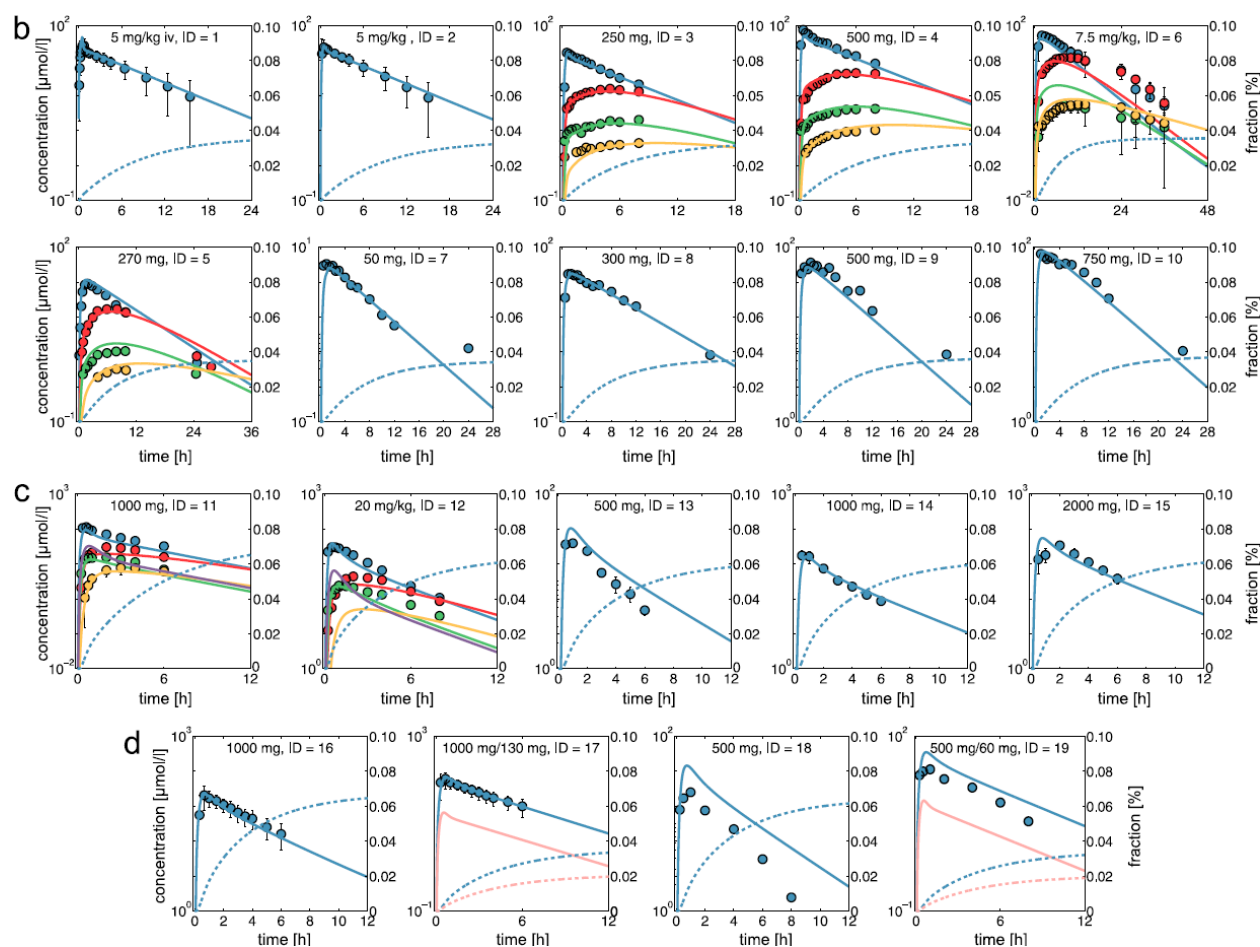


Figure 6: PBPK model development & validation for single doses of acetaminophen and caffeine. (a) Reaction diagram of twenty-one biochemical processes implemented in the PBPK models of APAP and CAF illustrating active drug transport (green), metabolizing reactions for phase I (purple) and phase II (yellow) metabolites, kidney plasma clearance (gray), and inhibition processes (red). Metabolic enzymes and transporters are shown next to the respective reaction. (b) PBPK model of CAF (CAF = blue, PX = red, TB = green, TP = yellow). (c) PBPK model of APAP (APAP = blue, APAPG = red, APAPC = green, APAPS = yellow, NAPQI = purple). (d) PBPK model for single administration of APAP and for co-administration of APAP and CAF (APAP = blue, CAF = pink). Simulated drug concentration-time curves (lines) were assessed with experimental PK profiles (circles). Renal excretion rates were additionally simulated for APAP and CAF (dashed lines). Study IDs and dose levels of the experimental data are shown within each plot (Supplementary Table S5).

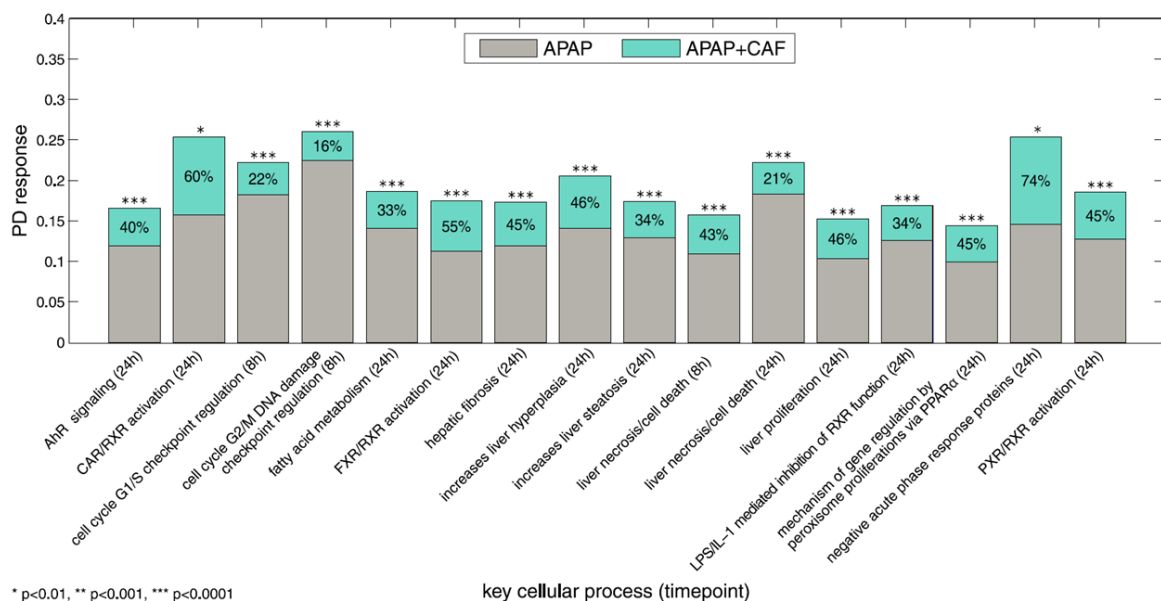


Figure 7: PD response of key cellular processes. PD responses for significantly perturbed key cellular processes following drug administration of APAP as single toxic dose (gray) or co-administered with CAF (mint green). Percentages indicate relative PD effects of CAF.

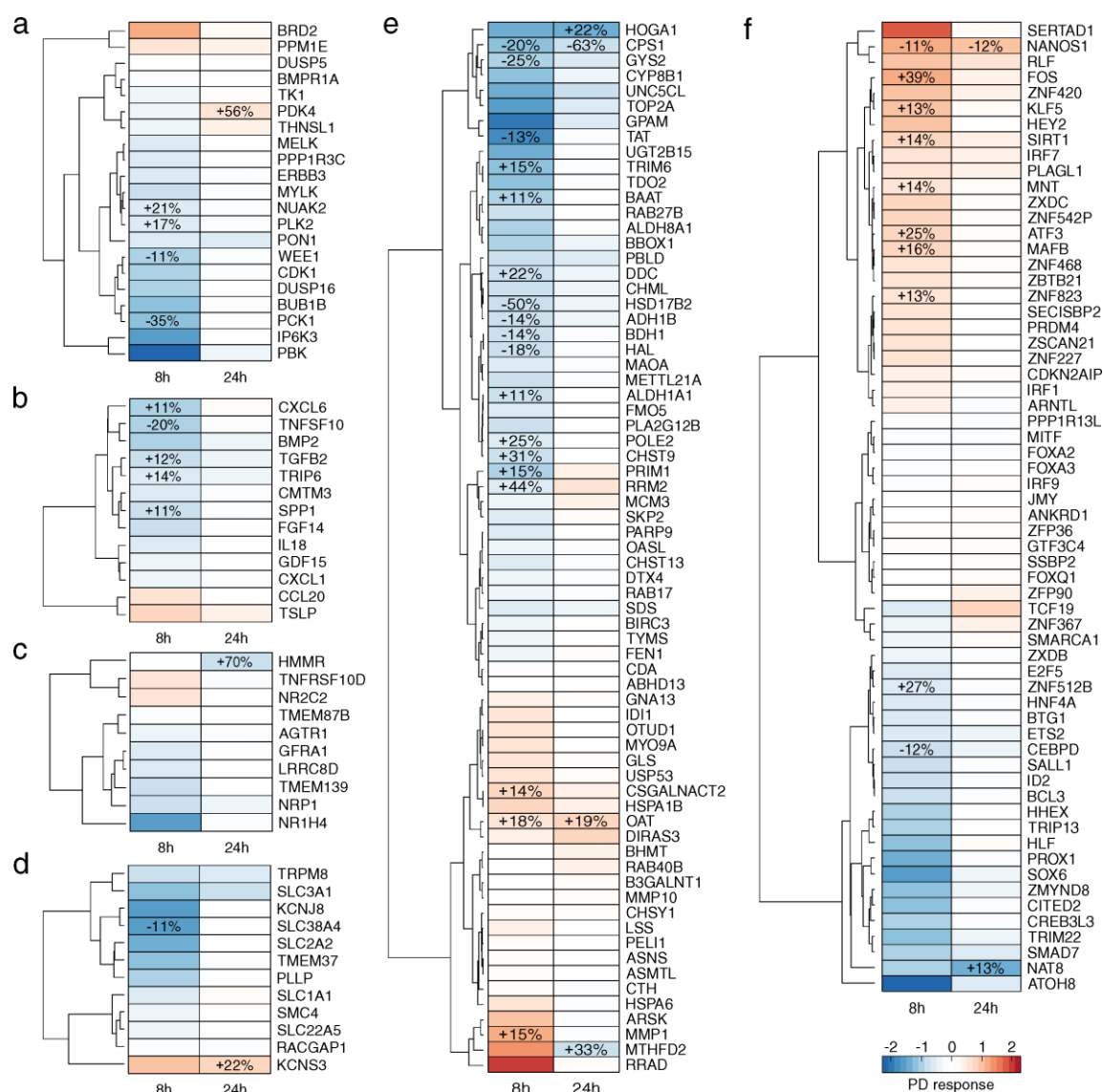


Figure 8: Pharmacodynamic (PD) response of individual genes. PD responses of significantly perturbed genes following drug administration of acetaminophen (APAP) as single toxic dose and correspondent PD effects induced by co-administration of caffeine (CAF). Genes were classified according to their functional classes. Relative PD effect values indicated by percentages were only shown for highly regulated genes (absolute fold change > 1.5 and absolute relative PD effect > 10%). (a) Kinase/Phosphatase. (b) Cytokine/Growth factor. (c) Receptor. (d) Ion channel/Transporter. (e) Metabolic enzyme. (f) Transcription/Translation regulator.

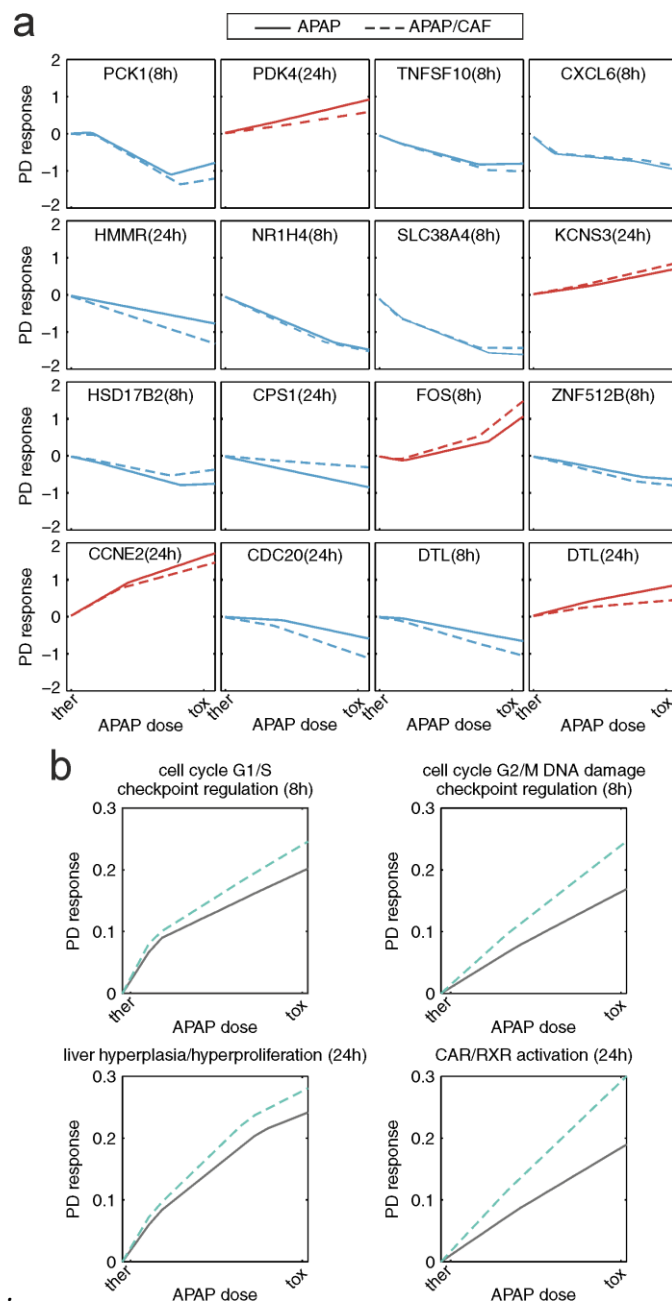


Figure 9: Dose escalation study. PD responses were predicted following single administration of APAP (solid lines) and co-administration of APAP and CAF (dashed lines). The doses were stepwise increased from therapeutic to toxic dose levels. (a) Individual genes. (b) Key cellular processes.

DIFFICULTIES

Though PICD is generally applicable, all results have been obtained by using transcriptomics data from the Open TG-GATEs library (Igarashi et al. 2015). The predictive accuracy will be further improved as soon as HeCaToS data become available.

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