SUPPLEMENTARY METHODS

Analysis of in vitro toxicity data

The analysis of time-series gene expression profiles from Open TG-GATEs (1) (ArrayExpress accession numbers: E-MTAB-798) including data pre-processing and normalization, differential expression analysis of single genes and overrepresentation analysis of key cellular processes were performed as explained before (2). Fold change values were calculated to indicate gene expression changes compared to time-matched controls. To represent key cellular processes, seventy-four hand-curated toxicity lists were extracted from QIAGENs Ingenuity Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity). Since primary human hepatocytes were analyzed (1), key cellular processes representing cardiac or renal toxicity were not considered. Genes with unknown functions were also not taken into account.

PBPK model development

PBPK models of APAP and CAF were built by use of the software PK-Sim® (version 6.0) and MoBi® (version 3.4) (Bayer Technology Services, GmbH, Leverkusen, Germany) (3,4), which are freely available for academic use (Supplementary Figure S1). Physicochemical drug properties of APAP, CAF and their metabolites were therefore obtained from the literature (Supplementary Table S1). Reference PBPK models were first developed and assessed by comparing simulated drug concentrations with clinical PK data from literature (5–9) (Supplementary Figure S2). Intestinal permeability values originally provided by PK-Sim® were slightly adjusted for APAP (1.9E-05 cm/min) and CAF (3E-05 cm/min) (Supplementary Figure S2). The standard distribution model of PK-Sim® was used to calculate partition coefficients and cellular permeabilities (3). The Michaelis-Menten constants (Km) and the maximal velocities (vmax) representing the kinetic behavior of active transport processes and metabolizing reactions were either taken from literature (10–16) or were fitted to best describe the experimental data (Supplementary Table S2). Relative abundance of relevant ADME enzymes and transporters (Supplementary Table S2) was estimated by using tissue-specific gene expression data (Supplementary Table S3) (17). Kidney plasma clearances were parametrized such that urinary excretion rates were in accordance with results observed in human clinical studies (18–20) (Supplementary Table S4). A competitive inhibition of CAF on CYP2E1 (13,21) and ABCB1 (19) with dissociation constants (Ki) of 48.5 µmol/l and 0.06 µmol/l, respectively, were modelled to consider the PK interaction of CAF on APAP (8,22). Respective reaction rates in the competitive inhibition processes were calculated as follows:

$$v = \frac{vmax * S}{Km * \left(1 + {}^{I}/_{Ki}\right) + S} \quad (1)$$

v = reaction rate, vmax = maximal reaction rate, S = free substrate (APAP) concentration, I = free inhibitor (CAF) concentration, Km = Michaelis-Menten constant in absence of the inhibitor.

The established reference PBPK models were further validated by using clinical PK data not used for model establishment (20,22–25) (Supplementary Figure S2) thereby leaving all model parameters unchanged, except parameters characterizing the specific design of the clinical studies (Supplementary Table S5). The model quality was evaluated by calculating normalized root-mean-square deviation (RMSD), coefficient of determination (R^2) values (2), and by comparing observed vs. predicted area under the curve values (AUCs) and maximal concentrations (cMax) of the different simulations.

Other system biology models for APAP

Several system biology models of APAP were published in literature and applied for different purposes such as toxicology (26–29) or pediatric scaling (30) (Supplementary Table S6). Here, a subset of five different models (26–30) is briefly explored (Supplementary Table S6) thereby focusing on (i) the underlying model structure including the modeling framework and the implemented biochemical processes, (ii) the clinical data used for model development and validation, and (iii) the modeling purpose & results. All models consider clearance processes of APAP and its metabolites, while Jiang et al. (30) and Ben-Shachar et al. (29) additionally considers several UGT and CYP enzymes for the metabolism of APAP (Supplementary Table S6). In our model active drug transport processes by ABCB1 and ABCG2 were additionally considered.

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