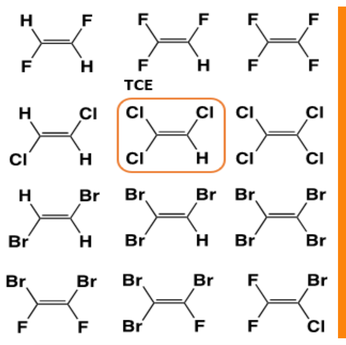
The Final EUTOXRISK Symposium 24



CS15: Multi-organ metabolism (MOM) of halogenated alkenes: trichloroethelene. The applied technology

Liliana Capinha, Yaran Zhang, Zofia Janstova, Anna-Katharina Ueckert, Marcel Leist, András Dinnyes, Jan Commandeur and Paul Jennings. University Konstanz, Biotalentum & Vrije Universiteit Amsterdam

Introduction

Trichloroethylene (TCE) is a high-production volume halogenated alkene that is currently a ubiquitous environmental pollutant. Occupational investigations and animal studies have demonstrated that TCE can cause various types of toxicity, including hepatotoxicity, nephrotoxicity and neurotoxicity.

Mechanistic *in vivo* and *in vitro* animal studies have shown that the hepatic and renal toxicities of TCE are not caused by the parent chemical but are the result of bioactivation to protein-reactive metabolites.

Nephrotoxicity from TCE has been attributed to the mercapturic acid pathway and is one of the major concerns from TCE exposure. This process starts at the hepatic level with glutathione (GSH) conjugation followed by a multi-step process in the proximal tubule nephron: (1) ϒ-glutamyl transpeptidase (brush border), (2) dipeptidase metabolism (brush border), (3) cysteine transport uptake, and (4) β-lyase metabolism to a toxic thioketene. Here, we investigated the effects of two human relevant regioisomers of GSH conjugates S-(1,2-dichlorovinyl)-glutathione and S-(2,2-dichlorovinyl)-glutathione, and their corresponding cysteine conjugates S-(1,2-dichlorovinyl)-cysteine and S-(2,2-dichlorovinyl)-cysteine in different cell specific *in vitro* systems. We investigated which GSTs are involved in TCE GSH conjugation; we followed the kinetics of these hepatic conjugates in a renal model (RPTEC/TERT1) and investigated the transcriptomic response from a 24-h exposure of a range of sub-cytotoxic concentrations in hepatic (HepaRG), renal (RPTEC/TERT1), endothelial (HUVEC/TERT2), and neuronal systems (iPSC derived).

Case study testing approach

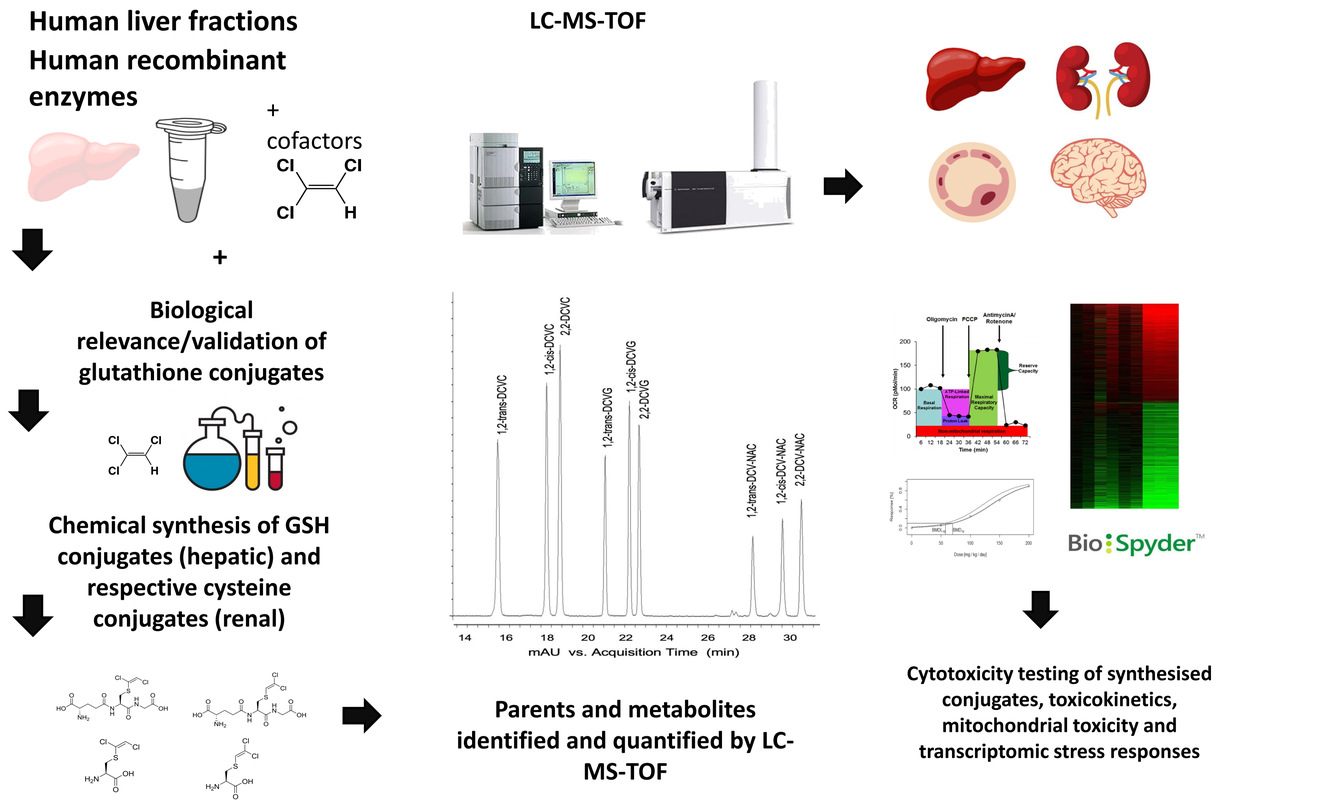


Fig 1. *CS15 – MOM – chemical testing workflow*

TCE hepatic GSH conjugation

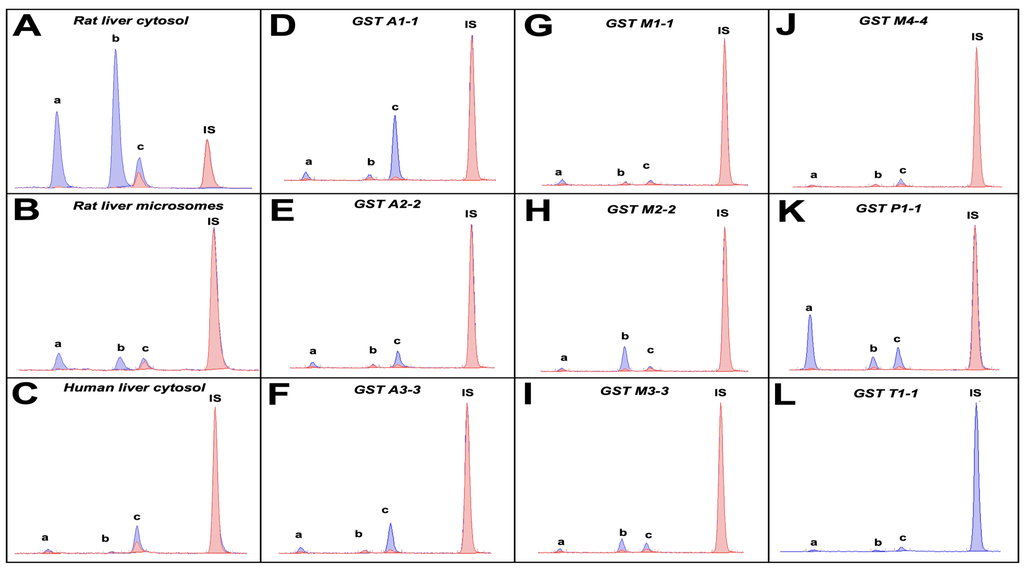


Fig 2*. Regioselectivity of GSH-conjugation of TCE by rat and human liver fractions and recombinant human GSTs a) 1,2 trans DCVG, b) 1,2 cis DCVG, c) 2,2 DCVG. IS: internal standard, blue: without GST inhibitor, red: with GST inhibitor.*

Renal kinetics of TCE-GSH conjugates

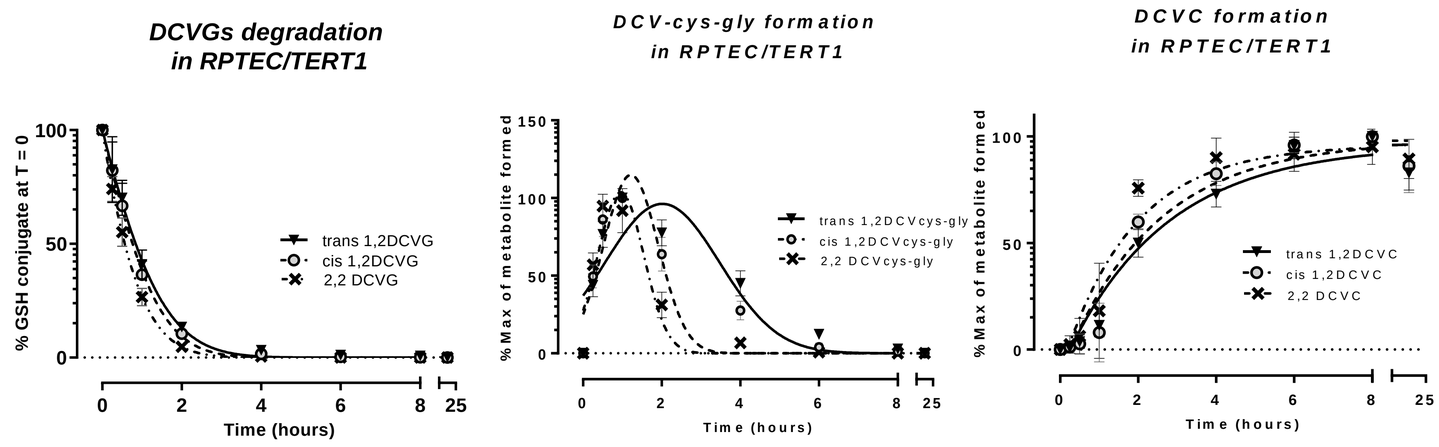
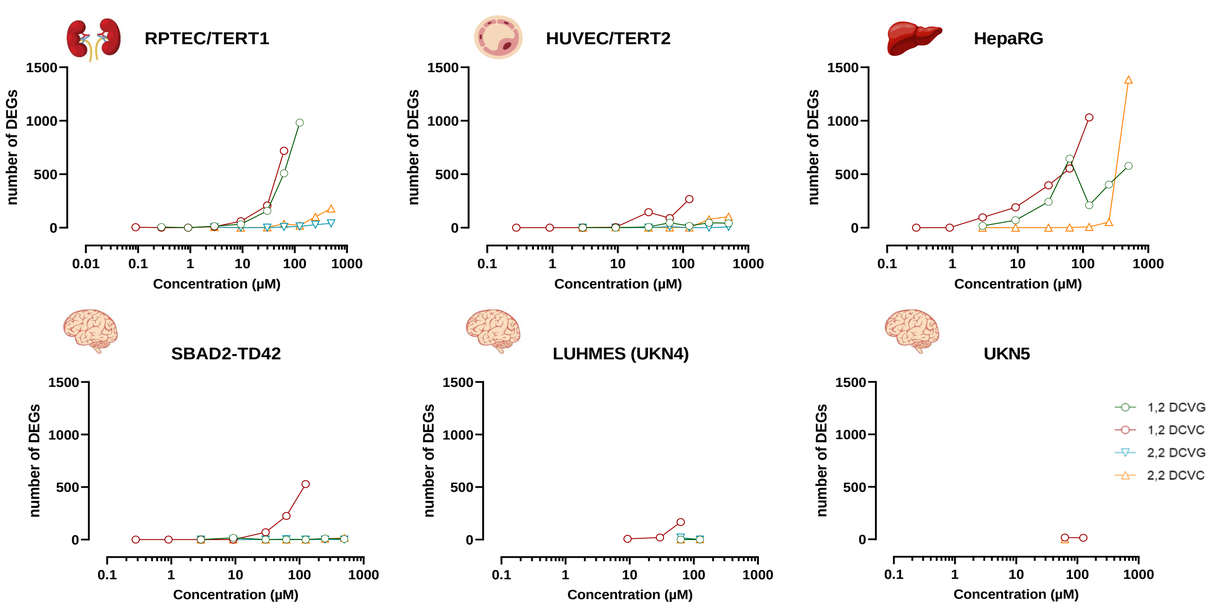
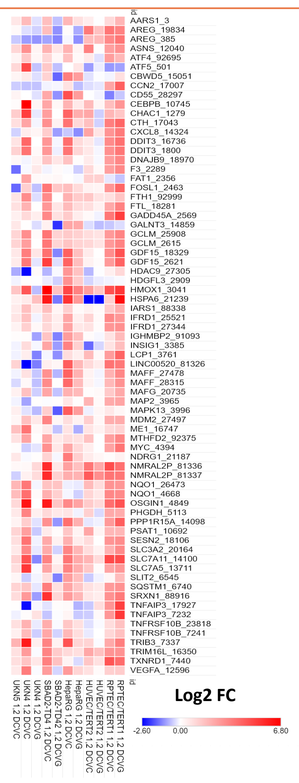


Fig 3. Biotransformation of 30 µM TCE-GSHs metabolites (1st order metabolites) over time in differentiated RPTEC/TERT1

Transcriptomic analysis of TCE conjugates





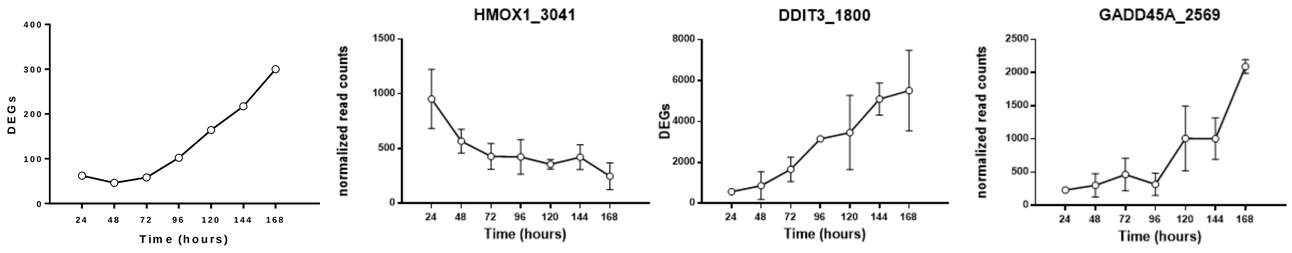
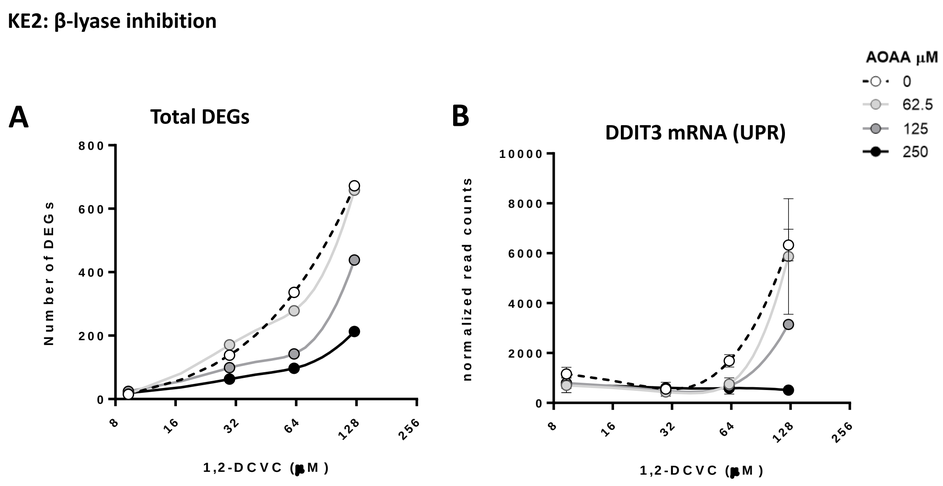


Fig. 5 *Seven day repeat dose treatment of highest sub-cytotoxic concentration of 1,2 DCVG in differentiated RPTEC/TERT1 .*



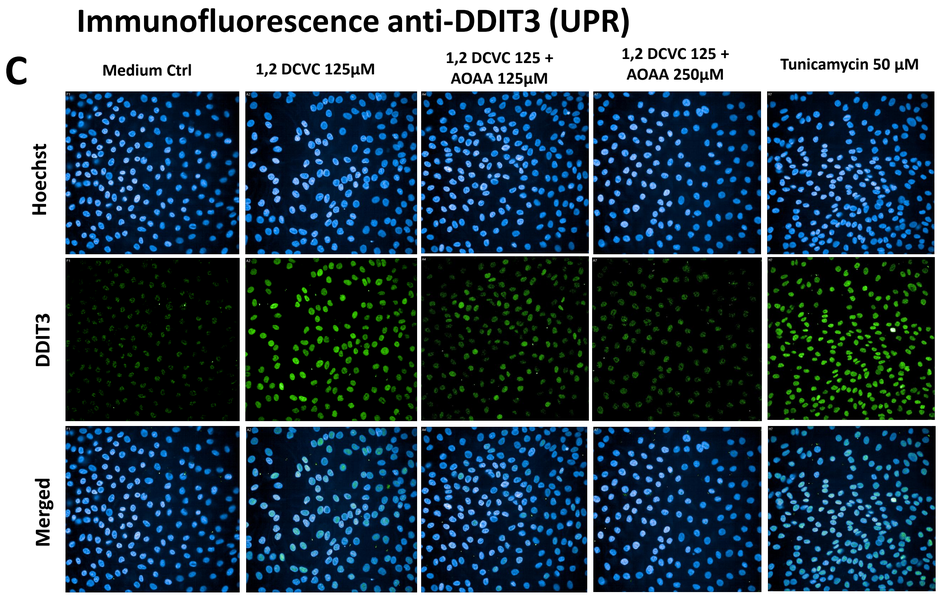


Fig 6. *Co-incubation of 1,2 DCVC and general β-lyase inhibitor - Aminooxyacetic acid (AOAA) in differentiated RPTEC/TERT1 A) Number of total DEGs B) Normalized read counts for DDIT3 mRNA C) Immunocytochemistry for anti-DDIT3*

The EU-ToxRisk project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002.

www.eu-toxrisk.eu/