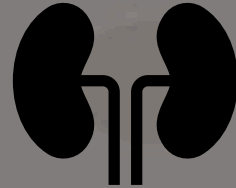
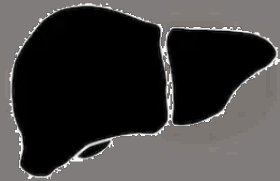
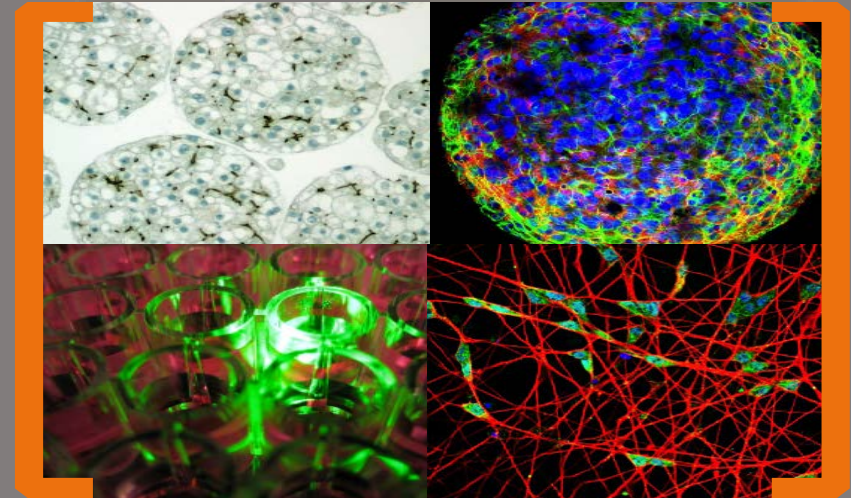


Brussels Nov 2021

Application of NAMs to detect multi-site metabolism



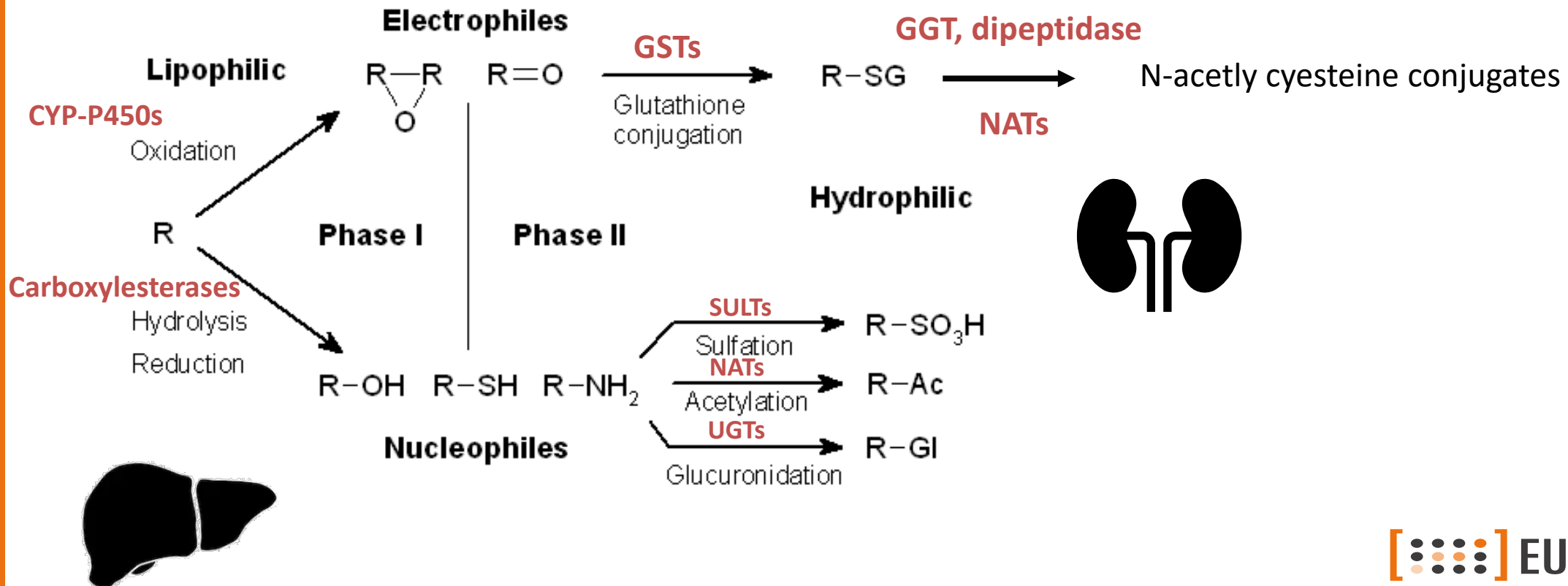
Prof. dr. Paul Jennings
Vrije Universiteit Amsterdam



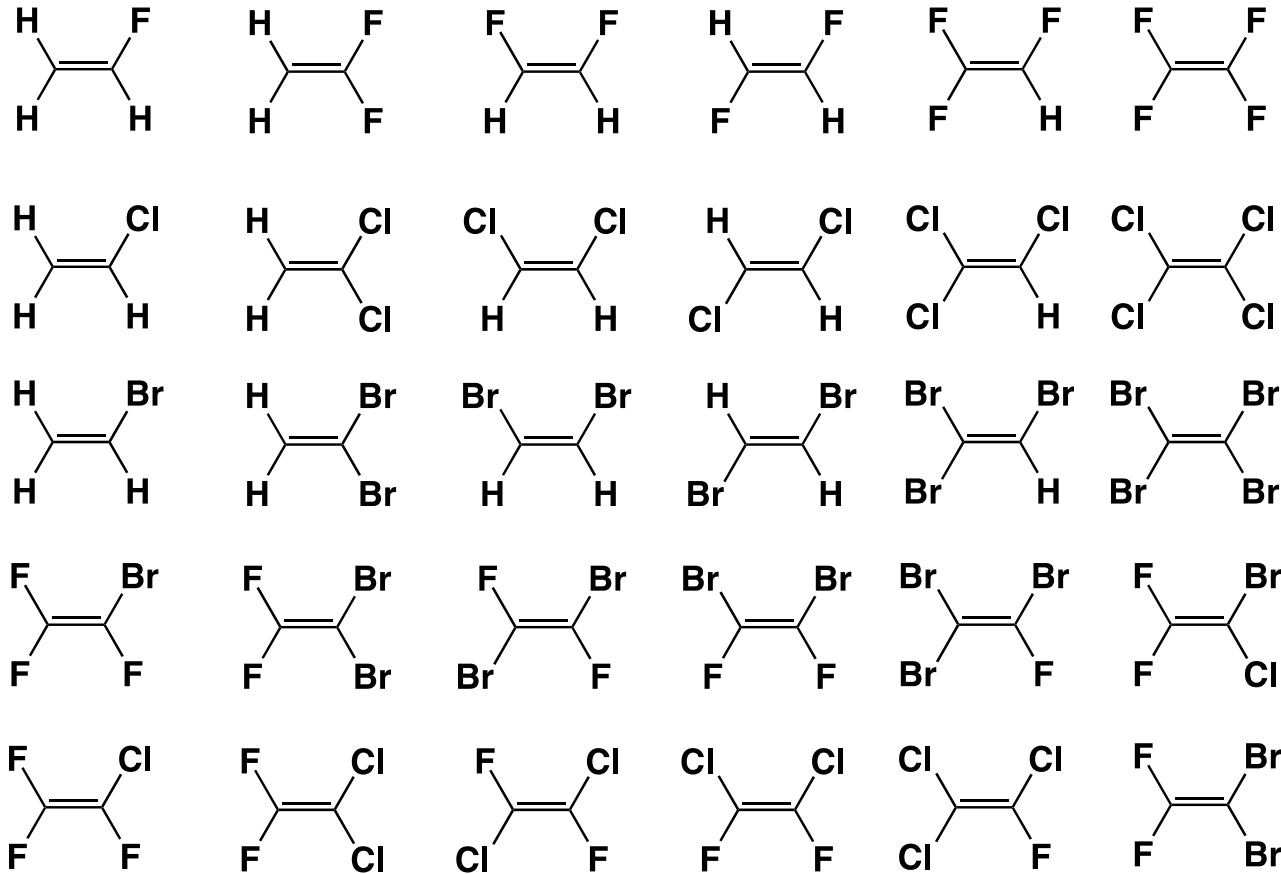
Xenobiotic metabolism

Phase I – Functionalization reactions: imply the creation of a functional group or the modification of an existing one in a drug.

Phase II – Conjugation reactions: imply coupling the compound to endogenous moieties (e.g., glucuronic acid, glutathione, sulfate, phosphate, amino acids, acetyl, etc.) to facilitate elimination in bile or urine



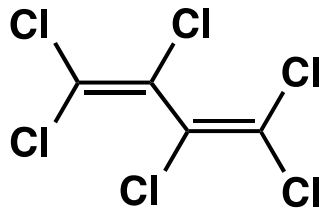
Halogenated Alkenes



- Organic solvents
- Degreasing agents
- Dry cleaning
- Synthetical intermediates
- Monomers polymer-synthesis
- inhalation anaesthetics

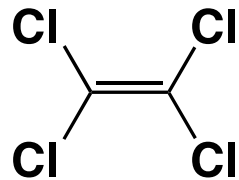
Chlorinated Alkenes

- Mainly industrial compounds used as organic solvents, degreasing agents and in dry cleaning.
- Produced in high tonnage per year.
- **Trichloroethylene** remains a top 10 priority chemical of concern for human health ([U.S. EPA, 2017](#)).



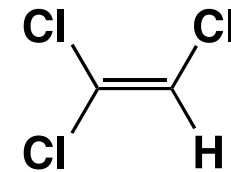
Hexachlorobutadiene (HCBD)

5 million kg / year



Tetrachloroethylene (PER)

77 million kg / year

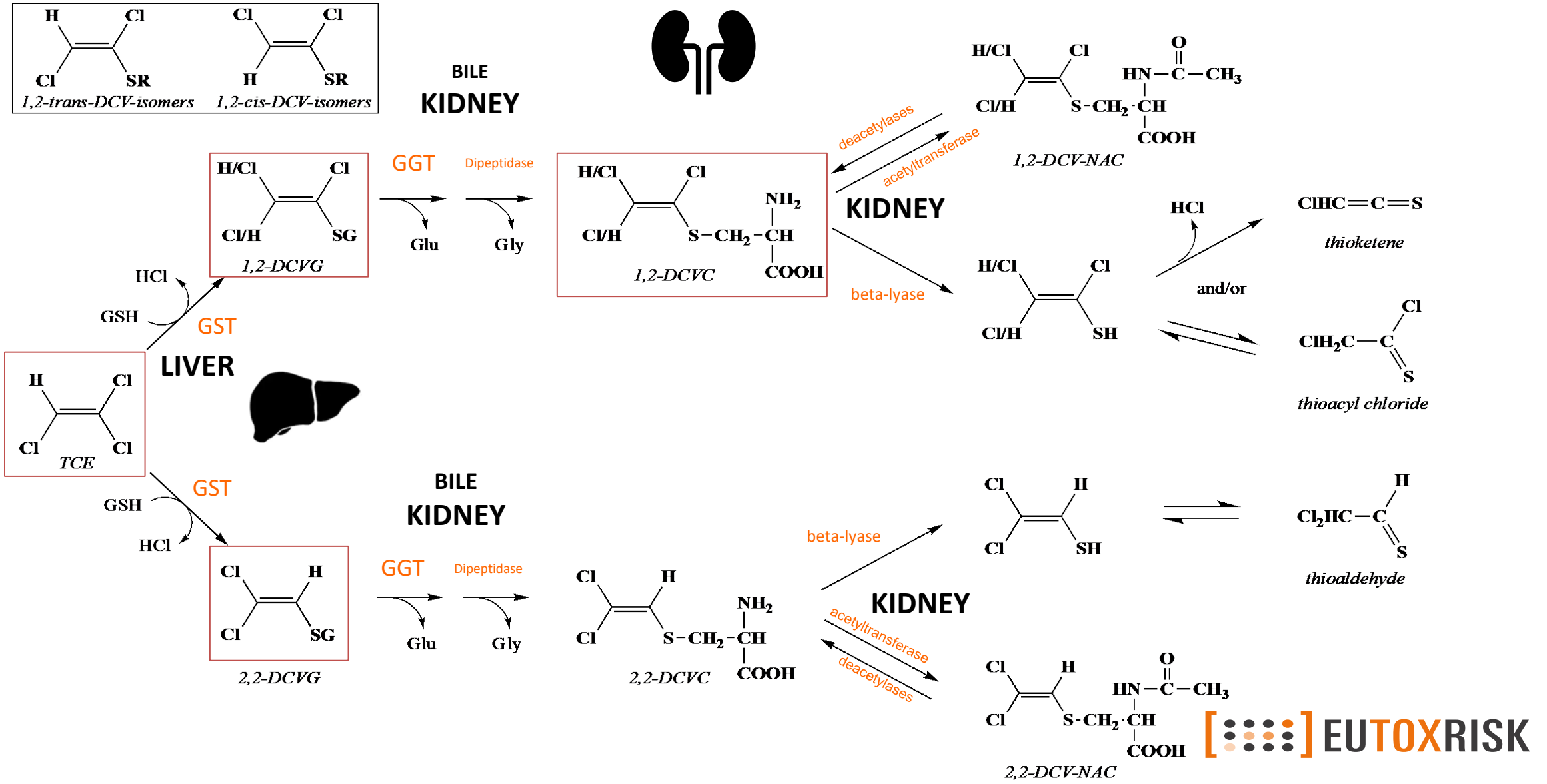


Trichloroethylene (TCE)

147 million kg / year

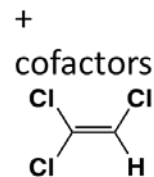
Toxic effects in rats: Mainly nephrotoxicity, renal carcinogenicity

Regioselective GSH-conjugation of Trichloroethylene (TCE)

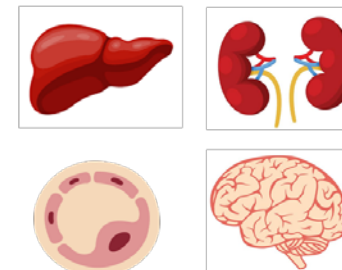


Workflow for testing compounds

Human liver fractions
Human recombinant enzymes



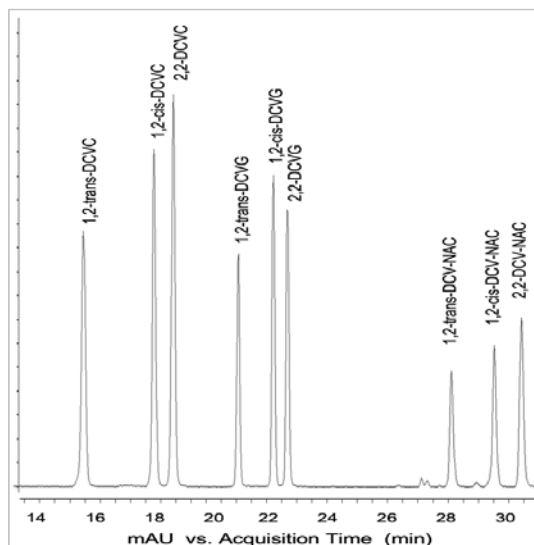
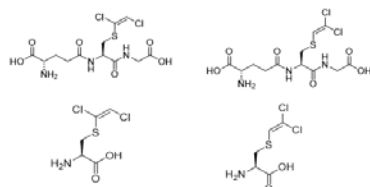
LC-MS-TOF



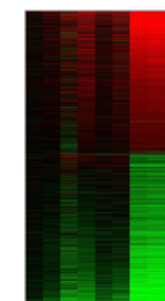
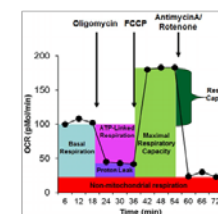
Biological relevance/validation of Glutathione conjugates



Chemical synthesis of GSH conjugates (hepatic) and respective cysteine conjugates (renal)



Parents and metabolites identified and quantified by LC-MS-TOF



BioSpyder™

Cytotoxicity testing of synthesised conjugates, toxicokinetics, mitochondrial toxicity and transcriptomic stress responses

Bioactivation of trichloroethylene to three regioisomeric glutathione conjugates by liver fractions and recombinant human glutathione transferases: Species differences and implications for human risk assessment



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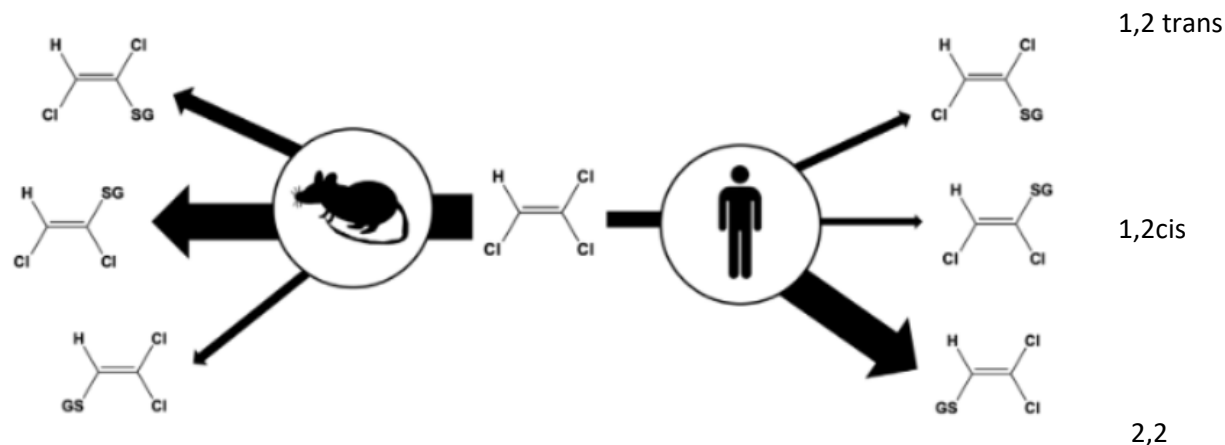
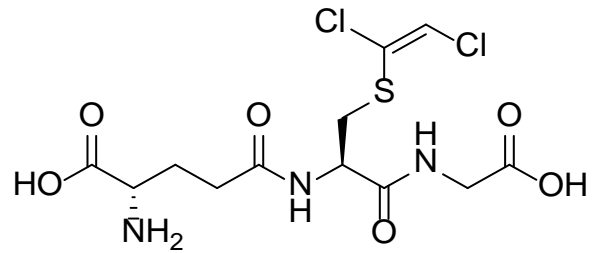


Table 1
Activities of rat and human liver fractions and recombinant human GSTs in GSH-conjugation of trichloroethylene.

Enzyme fraction	1,2-trans-DCVG	Specific activity (pmol/mg protein/hour) ^a		
		1,2-cis-DCVG	2,2-DCVG	Total DCVG
RLC	162 ± 4	230 ± 11	30 ± 3	422 ± 18
RLM	34 ± 3	23 ± 2	16 ± 2	73 ± 7
HLC	4 ± 1	N.D.	29 ± 4	33 ± 5
HLM	N.D.	N.D.	N.D.	N.D.
GSTA1-1	5 ± 1	2 ± 1	46 ± 5	55 ± 8
GSTA2-2	12 ± 3	N.D.	22 ± 4	34 ± 3
GSTA3-3	3 ± 1	N.D.	25 ± 6	28 ± 7
GSTM1-1	20 ± 5	N.D.	N.D.	20 ± 5
GSTM2-2	N.D.	41 ± 10	N.D.	41 ± 10
GSTM3-3	N.D.	12 ± 3	4 ± 2	16 ± 5
GSTM4-4	N.D.	N.D.	3 ± 1	3 ± 1
GSTP1-1	77 ± 8	18 ± 4	22 ± 5	117 ± 17
GSTK1-1	N.D.	N.D.	N.D.	N.D.
GSTT1-1	N.D.	N.D.	N.D.	N.D.
GSTT2-2	N.D.	N.D.	N.D.	N.D.

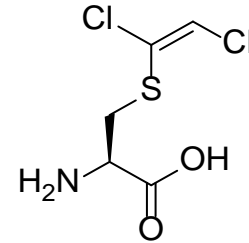
Trichloroethylene

1,2 DCVG



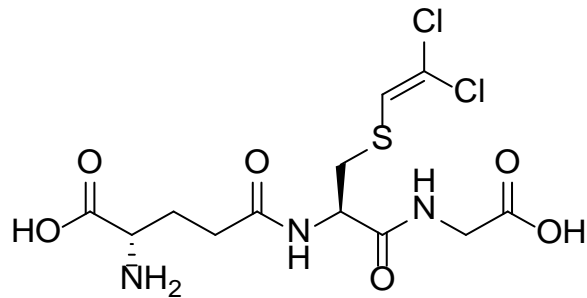
S-(1,2 *trans*-Dichlorovinyl)glutathione

1,2 DCVC



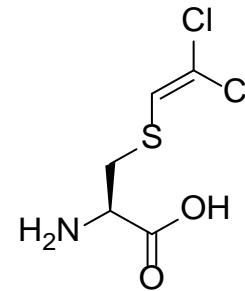
S-(1,2 *trans*-Dichlorovinyl)-L-cysteine

2,2 DCVG



S-(2,2-Dichlorovinyl)glutathione

2,2 DCVC



S-(2,2-Dichlorovinyl)-L-cysteine

Acknowledgements

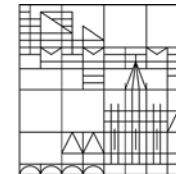


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