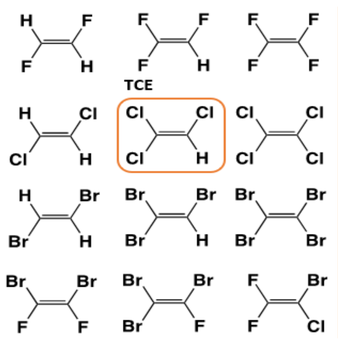
The Final EUTOXRISK Symposium 23



CS15: Multi-organ metabolism (MOM) of halogenated alkenes: trichloroethelene. Regulatory impact

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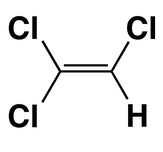
Background

Halogenated alkenes are widely used for various industrial applications, such as use as degreasers, pesticides, solvents and synthetic intermediates and for dry cleaning. Due to its high-volume production, trichloroethylene (TCE) is the best studied halogenated alkene. This is a man-made chemical that does not naturally occur in the environment and has been commonly used since the 1930s. Regulation of TCE by EPA began in 1980s and in 1990 Europe started to become concerned about its use for environmental, health and safety reasons. Multiple occupational and animal studies have demonstrated that TCE can cause various types of toxicity, including hepatotoxicity, nephrotoxicity and neurotoxicity in acutely and chronically exposed laboratory animals and humans.

Mechanistic *in vivo* and *in vitro* animal studies have demonstrated that the hepatic and renal toxicities of TCE are not caused by the parent compounds, but are the result of bioactivation to protein-reactive metabolites. This case study focused on the toxic effects from glutathione (GSH) conjugation (also known as mercapturic acid pathway) of TCE. This pathway has been demonstrated to result in nephrotoxicity, which starts with direct conjugation of TCE to GSH by hepatic GSH transferases (GSTs), followed by subsequent renal enzymatic steps leading to the formation of cysteine S-conjugates that are bioactivated by renal cysteine-conjugate β-lyases to unstable thiol compounds that rapidly rearrange to highly reactive products (figure 3). The mode of action of these halogenated alkenes were investigated *in vitro* using a complex workflow that allows the assessment of several key events that lead to nephrotoxicity from TCE and for a better understanding of hepatic and neurotoxicity from GSH conjugation of TCE, fundamental for the risk assessment of TCE in humans.

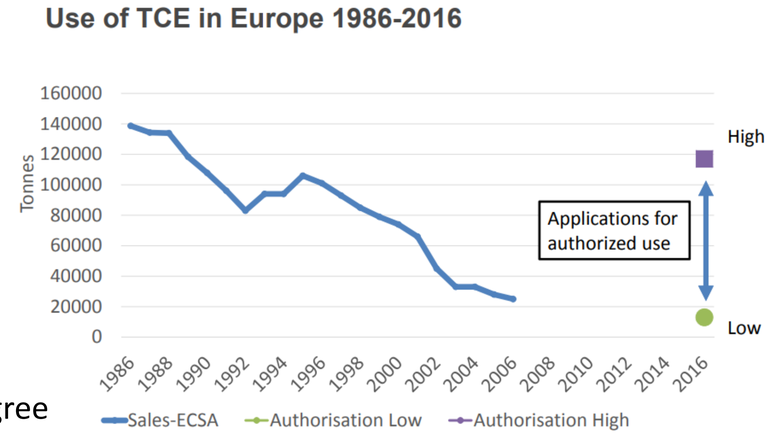
Trichloroethylene Infocard



C — Carcinogen

M — Suspected to be mutagenic

Ss — A majority of data submitters agree this substance is skin sensitising



April 21st 2016 EU 'Sunset date' – use of TCE banned in **Europe**, except for those with granted authorisation (outstanding) (ECHA).

January 14th 2021 (Trump), **USA** withdraws proposed ban of TCE (EPA).

Ubiquitous environmental pollutant.

Fig 1. *Trichloroethylene general information (source ECHA & EPA)*

TCE life cycle diagram

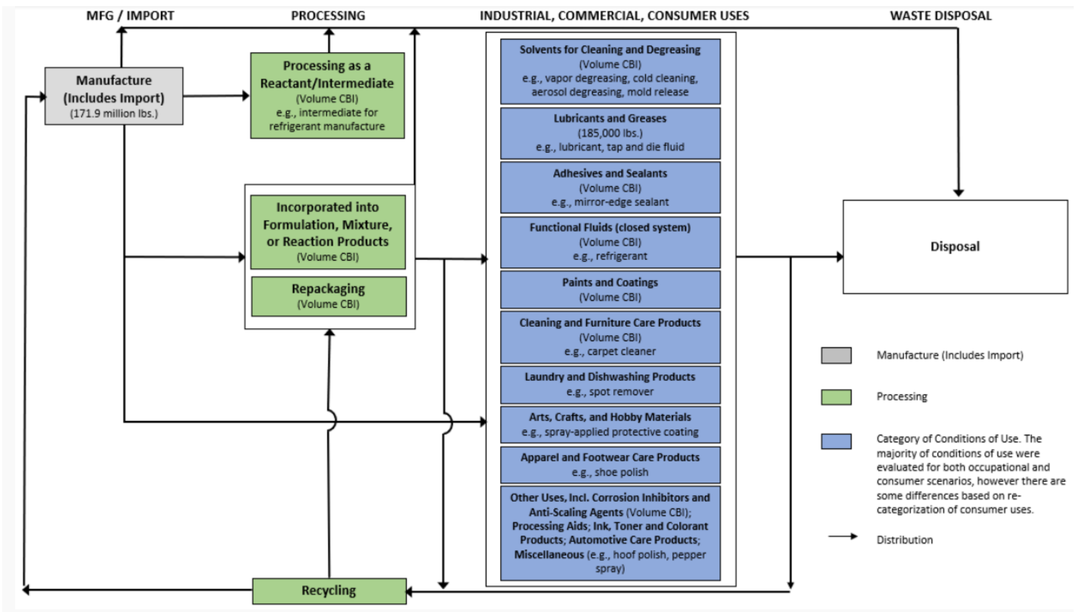


Fig 2. *TCE life cycle diagram (source: Trichloroethylene (TCE):Risk evaluation and Risk management under TSCA Section 6, January 26th, 2021, U.S. EPA*

Mercapturic acid pathway of TCE

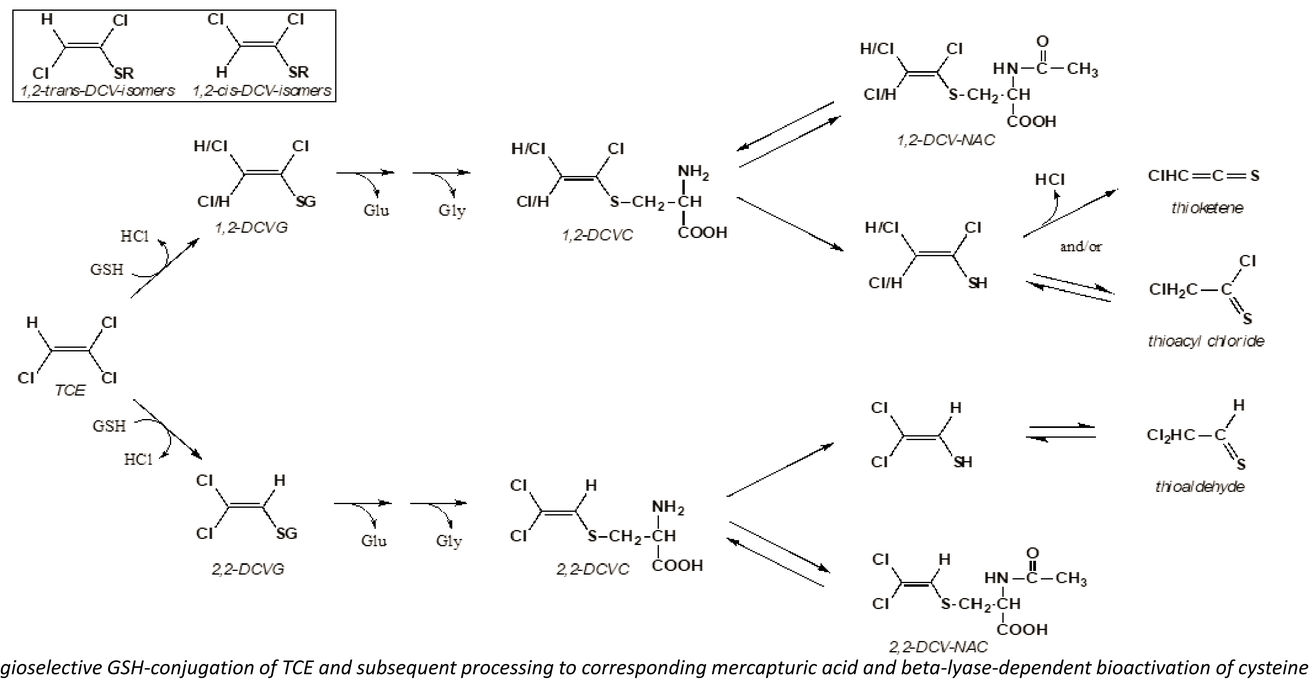


Fig 3. *Regioselective GSH-conjugation of TCE and subsequent processing to corresponding mercapturic acid and beta-lyase-dependent bioactivation of cysteine conjugates to reactive products.*

Adverse outcome pathway (AOP)

MIE	KE1	KE2	KE3	KE4	AO
GSH-conjugate	Cysteine-conjugate	β-lyase activity	Reactive metabolite	Mitotox/cell death	Kidney disease / renal cancer
• Analytical methods (LC-MS) for halogenated alkenes GSH conjugation identification • Liver microsomes and recombinant protein (GSTA, GSTM)	• Analytical methods (LC-MS) for halogenated alkenes cys-glycine conjugate & cysteine conjugate • GGT inhibition studies (Acivicin) / or utilisation of cells without GGT	• β-lyase inhibition studies (Aminooxyacetic acid) • Identification of quantifiable products of β-lyase activity (pyruvate, ammonia)	• Nrf2 (thiol reactivity) and UPR (proteotoxicity) induction (transcriptomics, temporal and repeat dose 5 days) • Differentially expressed proteins involved in Nrf2 pathway (HMOX1) and UPR (CHOP) with immunocytochemistry	• Mitostress assay by Seahorse bioanalyzer • Mitochondrial membrane potential studies (JC-1) • Cell viability (cell count, redox capacity)	

Outcome of the Case Study

Utilised NAMs could capture the MIE and KEs. Preventing KE1 (non-target cells) and KE2 (pharmacological inhibition) prevented subsequent events. Regioisomer specificity was also demonstrated where 1,2 was toxic and 2,2 was not. The developed IATA workflow was applied to other halogenated alkenes tetrachloroethylene and hexachlorobutadiene. It is expected that this IATA is applicable to other hepatic GSH metabolites with renal liabilities.

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www.eu-toxrisk.eu/