Cell Biol Toxicol (2015) 31:1–13
DOI 10.1007/s10565-015-9293-x

REVIEW ARTICLE

Three common pathways of nephrotoxicity induced by halogenated alkenes

Patrizia Cristofori · Aisha V. Sauer · Andrea Trevisan

Received: 28 July 2014 / Accepted: 29 January 2015 / Published online: 11 February 2015

Abstract Glutathione-dependent bioactivation is a common pathway in nephrotoxicity caused by haloalkanes and haloalkenes. Glutathione conjugation forms the link between halogenated hydrocarbons, based on the formation of an episulfonium ion (vicinal halomethanes) or a cysteine conjugate (haloalkenes). Herein, we review the metabolic pathways underlying the nephrotoxic effects of the three well-known haloalkenes trichloroethylene, tetrachloroethylene, and hexachloro-1:3-butadiene to emphasize the role of cysteine-conjugate β-lyase and the oxidative metabolism in renal toxicity. Activation by cysteine-conjugate β-lyase is the best-characterized mechanism causing toxicity due to haloalkene treatment in experimental models. However, the severity of toxicity differs considerably, with *S*-(1,2,2-trichlorovinyl)-L-cysteine being more toxic than *S*-(1,2-dichlorovinyl)-L-cysteine, which is in turn more toxic than *S*-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-cysteine. Moreover, two oxidative pathways involving cysteine *S*-conjugates (mediated by flavin-containing monooxigenase 3) and *N*-acetyl-L-cysteine conjugates (mediated by cytochrome P-450 3A) form derived sulfoxides, which represent alternative metabolites with toxic effects. In vitro and in vivo studies showed that sulfoxide metabolites are more toxic than cysteine-conjugate derivates. The cytochrome P-450 3A family, on the other hand, is sex specific, and its expression has only been reported in adult male rats and rabbits. In summary, haloalkenes are highly nephrotoxic in vivo and in vitro and their toxicity mechanisms are well documented experimentally. However, little information is available on their toxicity in humans, except for the carcinogenic effects established for high exposure levels of trichloroethylene and tetrachloroethylene.

Keywords Trichloroethylene · Tetrachloroethylene · Hexachloro-1:3-butadiene · Sulfoxide · β-Lyase · Nephrotoxicity

Introduction

An interesting feature of renal toxicology is the metabolic fate of certain chemicals, which are converted into nephrotoxic metabolites in the liver and subsequently disposed of in the kidney or directly activated in this organ (Dekant and Vamvakas 1996). The former metabolic pathway is mediated by glutathione (GSH) conjugation, giving rise to GSH conjugates and subsequently cysteine conjugates, by loss of glutamic acid and glycine. Acetylation into mercapturic acids is the final metabolic conjugation step in the kidney.

P. Cristofori
Pathology Department, GlaxoSmithKline, Ware, UK

A. V. Sauer
San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milan, Italy

A. Trevisan (✉)
Department of Cardiologic, Thoracic and Vascular Sciences, University of Padova, Via Giustiniani 2, 35128 Padova, Italy
e-mail: andrea.trevisan@unipd.it

Cysteine *S*-conjugates enter an alternative pathway in the kidney, which is mediated by cysteine-conjugate β-lyase (βL). Tateishi et al. (1978a, b) first used the term "cysteine-conjugate β-lyase" to define an enzyme catalyzing β-elimination from a cysteine *S*-conjugate. Subsequently, several pyridoxal 5′-phosphate (PLP) enzymes with βL activity have been identified in the kidney cell cytosol (Cooper et al. 2011 and references therein). βL activity was also demonstrated in rat kidney mitochondria (Cooper et al. 2001). PLP enzymes may be inhibited catalytically by covalent binding of by-products of βL reactions to PLP coenzymes (Adams et al. 2005). The final metabolites derived from this βL pathway are pyruvate, ammonia, and a chlorothioketene, which is responsible for the toxic and carcinogenic effects of halogenated alkenes in the kidney.

GSH-conjugate formation is the first step in the bioactivation of nephrotoxic halogenated alkenes (Elfarra and Anders 1984; Dekant 2003), and excretory processes play a pivotal role in the conversion of *S*-conjugates into their final metabolic products. In the kidney, βL activity leads to thioketene formation from cysteine *S*-conjugates (Elfarra and Anders 1984; Dekant et al. 1991), which causes nephrotoxicity in renal proximal tubule cells. Tubular cell damage is well established in the S_3 segment (*pars recta*) of the proximal tubule (Terracini and Parker 1965), although localization of βL can be detected along the entire proximal tubule (Kim et al. 1997; Trevisan et al. 1998).

As described for *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine, haloalkene *S*-cysteine derivates likely follow the "covalent binding hypothesis." Hence, a reactive metabolism intermediate might covalently modify lysyl ε-amino groups of neighboring proteins, thereby producing aberrant lysine-modified proteins (Nelson and Pearson 1990 and references therein). The altered protein conformation of these proteins may lead to the collapse of the inner mitochondrial membrane potential, changes in permeability, and cell death (James et al. 2002). Overall, *S*-conjugates strongly react with water, proteins, and nucleic acids (Dekant 2001).

Not only alkenes but also several alkanes, specifically halomethanes based on mustard formation via GSH (Rannug et al. 1978; Livesey et al. 1982; Koga et al. 1986), are nephrotoxic and mutagenic. In fact, βL-mediated metabolites were detected in the urine of subjects accidentally exposed to dihaloethanes (sulfur mustards) (Black and Read 1995).

The aim of this review is to summarize the toxic effect of cysteine conjugates derived from three selected halogenated alkenes on the kidney with regards to common metabolic pathways, not only related to βL activation. The reviewed haloalkenes trichloroethylene (TRI), tetrachloroethylene (TETRA), and hexachloro-1:3-butadiene (HCBD) are three-, four-, and hexa-chlorinated aliphatic hydrocarbons, which are relevant both in industrial and environmental settings.

Trichloroethylene

Trichloroethylene (TRI), also known as trichloroethene (CASRN 79-01-6), is a halogenated alkene widely used as industrial solvent and metal degreaser (30 % of total) Moreover, it is an intermediate product during fluorocarbon manufacturing (its main use) and a component of commercial stain removers, which are used for textile cleaning in laundries or drycleaners.

Metabolism

Figure 1 schematically summarizes the TRI metabolism. TRI is highly lipophilic and rapidly undergoes systemic distribution in animals and humans (Simmons et al. 2002). It is metabolized in the liver via cytochrome P-450 (Cyp450) 2E1 (Kim and Ghanayem 2006), as well as other Cyp450 isoforms (Nakajima et al. 1990). The first step is oxidation through an unstable epoxide into chloral and subsequently into trichloroethanol (TCE, about 30 %) or trichloroacetic acid (TCA, about 10 %), which are the main TRI metabolites in animals and humans (Lock and Reed 2006).

An alternative pathway, which operates in the kidney when the oxidative pathway becomes saturated, is via GSH conjugation to form *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) and then into other reactive metabolites via βL activity (Lock and Reed 2006). In humans, the GSH-mediated conversion probably accounts for <0.01 % of the administered TRI dose (Lock and Reed 2006). Whereas in mice, metabolites derived by oxidative metabolism are about 3600-fold greater than those derived from GSH conjugation (Kim et al. 2009).

The final step of the alternative pathway is acetylation of DCVC into *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (*N*-acDCVC), which is detectable in human urine (Bernauer et al. 1996). *N*-acDCVC is accumulated in the S_2–S_3 segments of the kidney by the organic anion

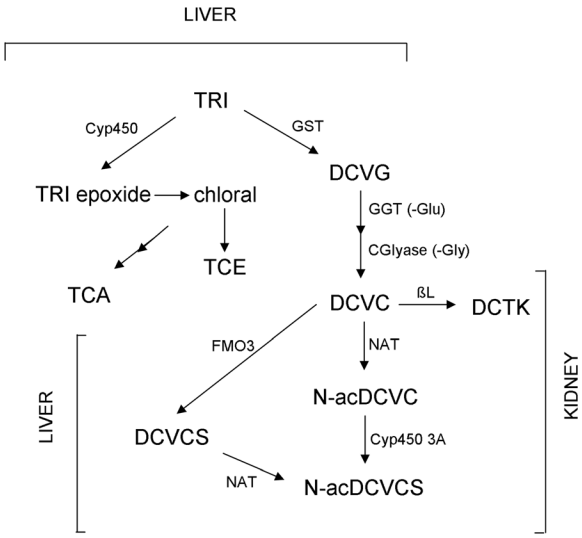

Fig. 1 TRI metabolism. Abbreviations: *TRI* trichloroethylene; *TCE* trichloroethanol; *TCA* trichloroacetic acid; *DCVG* *S*-(1,2-dichlorovinyl)-L-glutathione; *DCVC* *S*-(1,2-dichlorovinyl)-L-cysteine; *DCTK* dichlorothioketene; *N-acDCVC* *N*-acetyl-S-(1,2-dichlorovinyl)-L-cysteine; *DCVCS* *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide; *Cyp450* cytochrome P-450; *GST* glutathione *S*-transferases; *GGT* γ-glutamyltransferase; *CGlyase* cysteinylglicinase; *Glu* glutamic acid; *Gly* glycine; *βL* cysteine-conjugate β-lyase; *NAT* *N*-acetyltransferase; *FMO3* flavin-containing monooxigenase 3. Adapted from Barshteyn and Elfarra (2009)

system. Reaction with GSH produces not only mono-GSH but also di-GSH adducts as result of the addition of two molecules of GSH but elimination of only one molecule of hydrochloric acid (Irving et al. 2013).

Rat hepatic (not kidney) Cyp450 3A is further able to oxidize *N*-acDCVC to form *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (*N*-acDCVCS) (Werner et al. 1996). Alternatively, DCVC may be oxidized in the liver by flavin-containing monooxigenase 3 (FMO3) to form a Michael acceptor *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS) (Krause et al. 2003).

Toxicity

It is well established that several metabolisms are required for manifestation of TRI-induced toxicity (Elfarra 1997). TRI effects in the liver are attributed to the oxidative metabolism (Bull 2000), whereas renal effects are commonly associated with GSH conjugation (Lash et al. 2000).

Nephrotoxicity is commonly due to DCVC and well documented in rats or primary cultures of human kidney cells (Terracini and Parker 1965; Elfarra 1997). DCVC is activated by βL to form a chlorothioketene (Dekant et al. 1994; Lock and Reed 2006). Moreover, DCVCS formed via FMO3 is a reactive electrophile able to modify cellular molecules (Barshteyn and Elfarra 2009). The role of DCVCS in nephrotoxicity may partially be explained by an incomplete protection by aminooxiacetic acid (AOAA) (Lash et al. 1994). Recently, *N*-acDCVC and *N*-acDCVCS were shown to be similarly nephrotoxic in rats (Irving et al. 2013). These results differ from a previous study, in which *N*-acDCVCS was found to be more nephrotoxic in isolated rat kidney epithelial cells (Werner et al. 1996).

N-acDCVC and *N*-acDCVCS impact on the S_2–S_3 segments of the cortico-medullary proximal tubule, whereas DCVCS mainly affects the S_1–S_2 segments of the outer cortical area (Irving et al. 2013). De-acetylation into DCVC is one of the hypothetical mechanisms supporting *N*-acDCVCS nephrotoxicity, but *N*-acDCVCS also reacts with endogenous sulfhydryl-containing molecules that are directly involved in covalent modification of cortical kidney proteins (Irving et al. 2013). Moreover, sulfoxidation of DCVC into DCVCS is a bioactive process converting DCVCS into a more potent nephrotoxin than DCVC in rats (Lash et al. 1994).

Further, DCVC and DCV homocysteine reduce ATP concentrations intracellularly, thereby affecting thiol content and several energy-dependent processes, such as biosynthetic reactions, active transport, maintenance of cellular ions, and metabolite gradients (Jones et al. 1986). DCVC can directly act as Michael acceptor with cellular nucleophiles to form covalent adducts not only in proximal but also in distal tubules (Lash et al. 1994). Chronic TRI exposure causes cytomegaly, karyomegaly, and toxic nephrosis of tubular epithelial cells in the cortico-medullary junction in rats (Lock and Reed 2006). Transient effects on the kidney were also observed after an acute, nonfatal TRI exposure of a worker in a metal-degreasing machine (Carrieri et al. 2007).

Gender-related TRI toxicity was observed in rats, humans, and guinea pigs. Male rats were reported to be more sensitive to renal toxicity than female rats (Lash et al. 2001). After treatment with TRI, the excretion of TCA is significantly higher in female than in male guinea pigs, whereas no sex difference was observed in total TCA+TCE amount. This observation might be explained by the fact that constitutive expression of Cyp4502E1 in the liver is greater in female than in male

rats (Hibino et al. 2013). Finally, some findings in humans exposed to TRI suggest that women are more susceptible to kidney diseases and diabetes than men (Davis et al. 2005).

Genotoxicity and carcinogenicity

TRI was recently recognized as carcinogenic in humans (Guha et al. 2012) and has therefore been inserted in group 1 (previously group 2A) by the International Agency for Research on Cancer (IARC) (volume 106, in preparation). A positive correlation was found between TRI exposure and renal-cell carcinomas, whereas epidemiological evidence for an association between TRI exposure and non-Hodgkin's lymphoma or liver cancer was limited. In animals (mice and rats), TRI is a multisite carcinogen with an increased incidence of liver, kidney, lung, testes, and hematopoietic cancers (Guha et al. 2012).

The Environmental Protection Agency (2011) has not recognized TRI as genotoxic itself. However, recent research indicates that TRI-spiked water at high doses is mutagenic in the metabolic-activated Ames test (Tabrez and Ahmad 2012). Among TRI metabolites, chloral/chloral hydrate is genotoxic with and without metabolic activation, inducing mutations, chromosomal aberrations, micronuclei formation, and cell transformation (Rusyn et al. 2014 and references therein). DCVC Irving and Elfarra 2013), DCVCS (Daoud and Irving 1977), and *N*-acDCVC (Vamvakas et al. 1987; Birner et al. 1993) have strong, direct-acting mutagenic potential. TCA has no genotoxic properties, whereas dichloroacetic acid (DCA) shows only moderate evidence. No data are available on TCE and *S*-(1,2-dichlorovinyl)-L-glutathione (DCVG).

In addition to the potential genotoxic effects of TRI to induce kidney cancer, several non-genotoxic mechanisms such as (1) α_{2u}-globulin-associated nephropathy, (2) cytotoxicity not associated with α_{2u}-globulin, and (3) peroxisome proliferation activated receptor α (PPARα) activation have been described. There is no evidence that TRI directly causes α_{2u}-globulin accumulation or induces cell proliferation in rats. However, rats dosed with TCE accumulate hyaline droplets in the kidney with a dose-related incidence and severity (Green et al. 2003a). The second mechanism is due to the accumulation of toxic metabolites such as DCVC and DCVCS. In addition, cytotoxicity could be caused by formic acid formation from the oxidative metabolites TCA and TCE (Green et al. 2003a, b). Finally, TCE and its metabolites may activate PPARα and peroxisome proliferation in the kidney (Zhou and Waxman 1998; Goldsworthy and Popp 1987).

Regulatory context

Health assessment information for TRI was reviewed by the EPA (cfpub.epa.gov); the latest revision is dated September 28, 2011. Reference dose (RfD oral) and reference concentration (RfC inhalation) assessments are summarized in Tables 1 and 2, respectively.

Chronic RfD was estimated as a total of 0.0005 mg kg day^{-1}, whereas RfC was extrapolated route-by-route as 0.002 mg/m^3 (0.0004 ppm) using a physiologically based pharmacokinetics (PBPK) model.

In summary, TRI is carcinogenic by its mutagenic effect on the kidney. The lifetime exposure (0–70 years) risk levels are in the lower range of the concentration estimate in Table 3. Table 4 shows the route-to-route extrapolation of inhalation unit risks to oral slope factors for kidney cancer, non-Hodgkin's lymphoma, and liver cancer.

Tetrachloroethylene

Tetrachloroethylene (TETRA), also known as perchloroethylene or perchlorothene (CASRN 127-18-4), is one of the most widely used halogenated hydrocarbons. Similarly to TRI, TETRA is prevalently used as degreaser in metallurgy and as substitute of TRI in laundries and drycleaners (Trevisan et al. 2000).

Metabolism

Figure 2 schematically represents the TETRA metabolism. TETRA is poorly metabolized in the liver via Cyp450 2E1 into an epoxide (Dekant et al. 1987). The parent compound may also be directly GSH-conjugated to form *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC) and, via βL, is converted into a reactive thioketene (Lash and Parker 2001). The amounts of formed TCVC are relatively small, but higher than those formed by TRI exposure.

Similarly to TRI, the Cyp450 3A family and FMO3 are involved in the oxidation of TCVC and *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine (*N*-acTCVC) into the Michael acceptor *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide (TCVCS) (Krause et al. 2003) and *N*-acetyl-

Table 1 RfD for chronic oral exposure to TRI reviewed by the EPA (last revision September 28, 2011)

Critical effect	Point of departure	UF	Chronic RfD
Decreased thymus weight in female mice, 30-week drinking water study (Keil et al. 2009)	$HED_{99,LOAEL}$[a]: 0.048 mg kg day^{-1}	100	Candidate: 0.00048 mg kg day^{-1}
Decreased plaque-forming cell (PFC) response, increased delayed-type hypersensitivity in mice, drinking water exposure from gestation day 0 to 3 or 8 weeks of age (Peden-Adams et al. 2006)	LOAEL: 0.37 mg kg day^{-1}	1000	Candidate: 0.00037 mg kg day^{-1}
Increased fetal cardiac malformations in rats, drinking water exposure from gestation day 1–22 (Johnson et al. 2003)	$HED_{99,BMDL01}$[c]: 0.0051 mg kg day^{-1}	10	Candidate: 0.00051 mg kg day^{-1}
Multiple[a]	Multiple	Multiple	0.0005 mg kg day^{-1}

[a] The estimate supports an RfD of 0.0005 mg kg day^{-1} and reflects the midpoint among similar RfD candidates

[b] Ninety-ninth percentile human equivalent dose (HED) to lowest observed adverse effect level (LOAEL) per mouse (0.35 mg kg day^{-1}) using the internal dose metric of metabolized TRI/$kg^{3/4}$/day

[c] Ninety-ninth percentile HED for rat internal benchmark dose associated with a 1 % extra risk on a pup basis ($BMDL_{01}$) of 0.0142 mg TRI oxidized/$kg^{3/4}$/day

S-(1,2,2-trichlorovinyl)-L-cysteine (*N*-acTCVCS) (Guyton et al. 2014) in rat (Elfarra and Krause 2007) and rabbit liver microsomes (Werner et al. 1996; Ripp et al. 1999). In humans, the GSH metabolic pathway is relevant for the detection of the mercapturic acid *N*-acTCVC in exposed individuals (Birner et al. 1996).

Toxicity

Acute effects of TETRA on the liver and kidney have been reported in humans and animals. However, even high doses induce only minimal hepatic damage (Klaassen and Plaa 1966) caused by the oxidative metabolism (Buben and O'Flaherty 1985). After GSH conjugation, the TETRA metabolites are translocated to the kidney and (as the majority of haloalkenes) are further processed into nephrotoxic thioketene (Lash and Parker 2001).

The oxidation of TCVC into the reactive metabolite TCVCS causes a different kind of nephrotoxicity (Elfarra and Krause 2007), which, both in vivo and in vitro, is more potent than TCVC (Elfarra and Krause 2007).

Few studies are currently available, which assess gender differences in the TETRA metabolism. An in vitro study showed that the *S*-1,2,2-trichlorovinylglutathione (TCVG) formation rate was higher in tissue from male than that from female rats, thereby suggesting that this initial step contributes to the degree of renal injury and greater sensitivity of male rats to TETRA nephrotoxicity (Lash et al. 2002).

Table 2 RfC for chronic inhalation exposure to TRI reviewed by the EPA (last revision September 28, 2011)

Critical effect	Point of departure	UF	Chronic RfC
Decreased thymus weight in female mice, 30-week drinking water study (Keil et al. 2009), route-to-route extrapolation using PBPK model	$HED_{99,LOAEL}$[b]: 0.19 mg/m^3 (0.033 ppm)	100	Candidate: 0.0019 mg/m^3 (0.00033 ppm)
Increased fetal cardiac malformations in rats, drinking water exposure from gestation day 1–22 (Johnson et al. 2003), route-to-route extrapolation using PBPK model	$HED_{99,BMDL01}$[c]: 0.021 mg/m^3 (0.0037 ppm)	10	Candidate: 0.0021 mg/m^3 (0.00037 ppm)
Multiple[a]	Multiple	Multiple	0.002 mg/m^3 (0.0004 ppm)

[a] The estimate supports an RfD of 0.0005 mg kg day^{-1} and reflects the midpoint among similar RfD candidates

[b] Ninety-ninth percentile human equivalent dose (HED) to lowest observed adverse effect level (LOAEL) per mouse (0.35 mg kg day^{-1}) using the internal dose metric of metabolized TRI/$kg^{3/4}$/day

[c] Ninety-ninth percentile HED for rat internal benchmark dose associated with a 1 % extra risk on a pup basis ($BMDL_{01}$) of 0.0142 mg TRI oxidized/$kg^{3/4}$/day

Table 3 Drinking water concentrations at specific risk levels for TRI (source: EPA)

Risk level	Lower range of concentration estimate (μg/l)
1×10^{-4}	50
1×10^{-5}	5
1×10^{-6}	0.5

Genotoxicity and carcinogenicity

TETRA itself is not recognized as genotoxic (Bartsch et al. 1979). However, some metabolites such as TCVC (Vamvakas et al. 1989), TCVG (Vamvakas et al. 1989), and tetrachloroethylene oxide (Kline et al. 1982) induce genetic mutations in *Salmonella typhimurium*. In mice, TETRA has been reported to induce DNA damage in the liver but not in the kidney, hence suggesting an involvement in genotoxicity and carcinogenicity (Cederberg et al. 2010). In fact, TETRA causes hepatocellular carcinomas in mice and renal tubular cell carcinomas in rats (National Toxicology Program 1986).

Recently reviewed epidemiological studies on TETRA carcinogenicity (Guha et al. 2012; Vlaanderen et al. 2014) found a positive correlation between exposure and kidney, cervix, bladder, esophagus cancer, as well as non-Hodgkin's lymphoma. Furthermore, TETRA was shown to induce cancer in the hematopoietic system, testes, kidney, and brain of rats (Guha et al. 2012). TETRA was therefore considered as likely carcinogenic to humans (Guha et al. 2012), and its classification in group 2A was confirmed (International Agency for Research on Cancer 1995).

Regulatory context

Health assessment information was reviewed by the EPA (cfpub.epa.gov); the latest revision is dated October 2, 2012. RfD and RfC assessments for TETRA are summarized in Tables 5 and 6, respectively.

Chronic RfD was estimated as 0.006 mg kg day^{-1}, whereas RfC was extrapolated route-by-route as 0.04 mg/m^3 using a PBPK model.

In summary, TETRA is carcinogenic by its mutagenic effect on several organs and tissues. The lifetime exposure (0–70 years) risk levels are in the lower range of the concentration estimate in Table 7 for oral and Table 8 for chronic inhalation exposure. Data in Table 8 are from a multistage model with linear extrapolation from the point of departure ($BMCL_{10}$), followed by extrapolation to humans using the PBPK model of Chiu and Ginsberg (2011). Table 9 shows the estimated dose–response incidence of hepatocellular carcinomas in male mice after TETRA inhalation (EPA, by Japan Industrial Safety Association 1993).

Hexachloro-1:3-butadiene

HCBD is an olefin (CASRN 87-68.3) prevalently obtained as by-product during the manufacturing of chlorinated solvents and is also used as a vineyard fumigant. HCBD is detectable in ambient and drinking water, in aquatic organism, birds, and mammals indicating bioaccumulation. However, no biomagnification was observed (World Health Organization 1994).

Metabolism

Figure 3 shows the schematic representation of the HCBD metabolism. HCBD is GSH-conjugated by liver glutathione *S*-transferases (GST) to form *S*-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-glutathione (PCBG), which is then removed to the bile, reabsorbed by the intestine, and subsequently translocated to the kidney (Birner et al. 1998). In the kidney, PCBG is metabolized by renal γ-glutamyltransferase (GGT) and

Table 4 Route-to-route extrapolation of site-specific inhalation unit risks to oral slope factors (source: EPA)

	Kidney	Non-Hodgkin'S lymphoma	Liver
Inhalation unit risk (risk per ppm)	5.49×10^{-3}	1.10×10^{-2}	5.49×10^{-3}
ppm per mg kg day^{-1}	1.70	1.97	2.82
Oral slope factor (risk per mg kg day^{-1})	9.33×10^{-3}	2.16×10^{-2}	1.55×10^{-2}

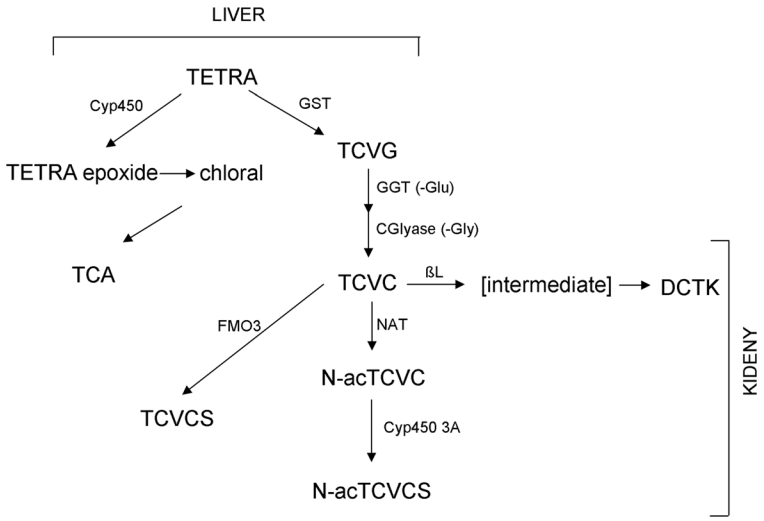

Fig. 2 TETRA metabolism. Abbreviations: *TETRA* tetrachloroethylene; *TCA* trichloroacetic acid; *TCVG* *S*-(1,2-trichlorovinyl)-L-glutathione; *TCVC* *S*-(1,2-trichlorovinyl)-L-cysteine; *DCTK* dichlorothioketene; *N-acTCVC* *N*-acetyl-*S*-(1,2-trichlorovinyl)-L-cysteine; *TCVCS* *S*-(1,2-trichlorovinyl)-L-cysteine sulfoxide; *Cyp450* cytochrome P-450; *GST* glutathione *S*-transferases; *GGT* γ-glutamyltransferase; *CGlyase* cysteinylglicinase; *Glu* glutamic acid; *Gly* glycine; *βL* cysteine-conjugate β-lyase; *NAT* *N*-acetyltransferase; *FMO3* flavin-containing monooxigenase 3. Adapted from Guyton et al. (2014)

cysteinglycinase into *S*-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-cysteine (PCBC). Its acetylation produces the final metabolite *N*-acetyl-*S*-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-cysteine (*N*-acPCBC). Both PCBC and *N*-acPCBC are accumulated by the organic anion transport system in the kidney. The former may be cleaved by renal βL, producing a reactive thioketene with nephrotoxic effects, whereas the latter is excreted into the urine. Two diastereomers, (*R*) and (*S*)-*N*-acPCBCS, respectively, have also been reported, but only (*S*) diastereomer is conjugated to GSH (Birner et al. 1998).

In male rats, the predominantly produced metabolite is *N*-acetyl-*S*-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-cysteine sulfoxide (*N*-acPCBCS) derived from the sex-specific oxidation of *N*-acPCBC by Cyp450 3A1/2 (Birner et al. 1998).

Toxicity

Insufficient data are available on HCBD toxicity in humans. However, in general, each metabolic step is an order of magnitude lower in humans than in rats (Green et al. 2003b).

Several experimental studies have been conducted to assess HCBD toxicity in animals. Ishmael et al. (1982) established that HCBD causes necrosis of the *pars recta* of the proximal tubule in the kidney. This initial observation was confirmed by several other studies (Trevisan et al.

Table 5 RfD for chronic oral exposure to TETRA reviewed by the EPA (last revision October 2, 2012)

Critical effect	Point of departure[b]	UF	Chronic RfD
Neurotoxicity (reaction time, cognitive effects) in occupationally exposed adults (Echeverria et al. 1995)	LOAEL: 9.7 mg kg day^{-1}	1000	Candidate: 0.0097 mg kg day^{-1}
Neurotoxicity (color vision) in occupationally exposed adults (Cavalleri et al. 1994)	LOAEL: 2.6 mg kg day^{-1}	1000	Candidate: 0.00026 mg kg day^{-1}
Multiple[a]	Multiple	Multiple	0.006 mg kg day^{-1}

[a] Midpoint supported by the two principal studies

[b] Route-to-route extrapolation from inhalation exposure using PBPK model (Chiu and Ginsberg 2011)

Table 6 RfC for chronic inhalation exposure to TETRA reviewed by the EPA (last revision October 2, 2012)

Critical effect	Point of departure	UF	Chronic RfC
Neurotoxicity (reaction time, cognitive effects) in occupationally exposed adults (Echeverria et al. 1995)	LOAEL: 56 mg/m^3	1000	Candidate: 0.056 mg/m^3
Neurotoxicity (color vision) in occupationally exposed adults (Cavalleri et al. 1994)	LOAEL: 15 mg/m^3	1000	Candidate: 0.015 mg/m^3
Multiple[a]	Multiple	Multiple	0.04 mg/m^3

[a] Midpoint supported by the two principal studies

1998, 1999, 2001; Cristofori et al. 2007; Maguire et al. 2013). Transcriptome studies showed a dose-dependent down-regulation of regucalcin, a calcium-binding protein (Chiusolo et al. 2008) and an up-regulation of kidney injury molecule-1 (KIM-1), a type I cell membrane protein (Chiusolo et al. 2010). In young adult rats, the accumulation of hyaline droplets was observed in tubular cells independently from the administered dose (Birner et al. 1995; Pähler et al. 1997; Cristofori et al. 2013).

HCBD reduces *p*-aminohippurate accumulation (Berndt and Mehendale 1979) and induces lipid peroxidation in renal cells (Sadeghnia et al. 2013). According to the metabolic pathway of halogenated alkenes into nephrotoxic thioketenes, nephrotoxicity of HCBD is commonly attributed to PCBC. Similarly to TRI and TETRA metabolisms, *N*-acPCBCS is directly cytotoxic for proximal tubular cells (Birner et al. 1998). This effect is independent from βL activation, as demonstrated by cytotoxicity also in the presence of a βL inhibitor such as AOAA (Birner et al. 1995). Interestingly, administration of low doses of HCBD to mildly hyperoxaluric rats induces crystalluria (Gambaro et al. 2006).

Studies on the sex-related nephrotoxicity of HCBD have remained inconclusive. Birner et al. (1995) found that HCBD-induced renal effects are more severe in male than in female rats in accordance with sex-specific sulfoxide metabolite formation. Whereas other authors found that female rats were about four times more sensitive to HCB than male rats (Hook et al. 1983; Kuo and Hook 1983; Ishmael and Lock 1986; Trevisan et al. 2005). These findings might be due to differences in hepatic and renal enzymes, which are responsible for the detoxification and/or activation of HCBD. In particular, female rat kidneys show a marked dose-related decrease in non-protein sulfhydryl content (Hook et al. 1983). Consistently, HCBD is more and earlier nephrotoxic in female than male rats (Trevisan et al. 2005), while aging does not increase susceptibility (Zanetti et al. 2010). No sex-dependent differences in nephrotoxicity were observed in mice (Lock et al. 1984).

Genotoxicity and carcinogenicity

Chlorovynyl-substituted cysteine conjugates are mutagenic in the Ames test even in the absence of activating enzymes (Dekant et al. 1986), whereas HCBD itself is not recognized as genotoxic (reviewed in Dekant et al. 1990). IARC classified HCBD in group 3 for not having a recognized carcinogenic potential in humans or animals (International Agency for Research on Cancer 1999). However, available research indicates that ade-

Table 7 Drinking water concentrations at specific risk levels for TETRA (EPA)

Risk level	Lower range of concentration estimate (μg/l)
1×10^{-4}	2000
1×10^{-5}	200
1×10^{-6}	20

Unit risk assuming 2 l of water consumption/day by a 70-kg human

Table 8 Air concentration at specific risk levels for TETRA (EPA)

Risk level	Lower range of concentration estimate (μg/m^3)
1×10^{-4}	400
1×10^{-5}	40
1×10^{-6}	4

Multistage model with linear extrapolation from the point of departure [benchmark concentration associated with a 10 % extra risk ($BMCL_{10}$)], followed by extrapolation to humans using the PBPK model (Chiu and Ginsberg 2011)

Table 9 Estimated dose–response incidence for hepatocellular carcinomas in male mice by TETRA inhalation (EPA, Japan Industrial Safety Association 1993)

Concentration (ppm)	Total liver oxidative metabolism (mg $kg^{-0.75}$ day^{-1})	Tumor incidence
0	0	13/46
10	2.25	21/49
50	8.25	19/48
250	33.6	40/49

nomas and adenocarcinomas of the kidney are induced by this olefin (Kociba et al. 1977). Hence, HCBD was reviewed by the EPA for its carcinogenic potential (see next section and Tables 10 and 11).

Regulatory context

Health assessment information was reviewed by the EPA (cfpub.epa.gov); the latest revision for RfD assessment (status withdrawn) is dated January 5, 1993. No data are available for RfC assessment. The carcinogenicity assessment was revised on January 4, 1991 and is summarized in Tables 10 and 11.

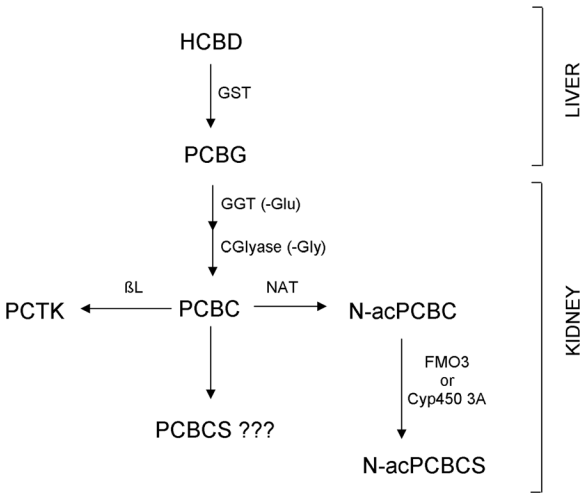

Fig. 3 HCBD metabolism. Abbreviations: *HCBD* hexachloro-1:3-butadiene; *PCBG S*-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-glutathione; *PCBC S*-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-cysteine; *PCTK* pentachlorothioketene; *N-acPCBC N*-acetyl-S-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-cysteine; *PCBCS S*-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-cysteine sulfoxide; *N-acPCBCS N*-acetyl-*S*-(1,2,3,4,4-pentachloro-1:3-butadienyl)-L-cysteine sulfoxide; *Cyp450* cytochrome P-450; *GST* glutathione *S*-transferases; *GGT* γ-glutamyltransferase; *CGlyase* cysteinylglycinase; *Glu* glutamic acid; *Gly* glycine; *βL* cysteine-conjugate β-lyase; *NAT N*-acetyltransferase, *FMO3*flavin-containing monooxigenase 3. Adapted from Birner et al. (1998)

Table 10 Drinking water concentrations at specific risk levels for HCBD: data for carcinogenic risk after oral exposure (EPA)

Risk level	Concentration (μg/l)
1×10^{-4}	50
1×10^{-5}	5
1×10^{-6}	0.5

Concluding remarks

GSH-dependent bioactivation is a common pathway of nephrotoxicity caused by haloalkanes (Guengerich 2003) and haloalkenes (Anders 2004). GSH conjugation is the common link between halogenated hydrocarbons with the formation of an episulfonium ion as possible fate of vicinal halomethanes and cysteine conjugate formation by βL-mediated cleavage of haloalkenes (Anders 2004).

The aims of this review were to summarize the metabolic pathways involved in the nephrotoxic effects of the three well-known haloalkenes TRI, TETRA, and HCBD and to emphasize the role of βL and the oxidative metabolism for renal toxicity.

"Lethal cleavage" by βL is the best-characterized event underlying the toxic effects of haloalkenes in experimental settings. However, the severity of toxicity may vary considerably. As assessed by histopathological examination, TCVC is more toxic than DCVC, which in turn is more toxic than PCBC. These results correlated with an increase in mercapturic acid secretion and toxicity was more pronounced in male rats (Birner et al. 1997). Moreover, two alternative oxidative pathways involving cysteine *S*-conjugates (mediated by FMO3) and *N*-acetyl-*S*-conjugates (mediated by

Table 11 Dose–response data (carcinogenic oral exposure) for renal tubular adenomas and adenocarcinomas in rats (Kociba et al. 1977)

mg kg day^{-1}	HED (mg kg day^{-1})	Tumor incidence
0	0	1/90
0.2	0.04	0/40
2.0	0.4	0/40
20.0	4.0	9/39

Cyp450 3A) form derived sulfoxides, which represent additional factors underlying toxic effects. Among the three haloalkenes, the TRI and TETRA metabolisms form sulfoxides both through DCVC (or TCVC) and *N*-acDCVC (*N*-acTCVC), whereas HCBD forms only *N*-acPCBDS. It is important to note that sulfoxide metabolites were shown both in in vitro and in vivo studies to be more toxic than cysteine-conjugates derivates (Birner et al. 1998; Elfarra and Krause 2007; Irving et al. 2013). The Cyp450 3A family, on the other hand, is sex specific and its expression has been reported in adult male rats (Werner et al. 1995) and rabbits only (Ripp et al. 1999).

In conclusion, haloalkenes are highly nephrotoxic in vivo and in vitro, based on three common metabolic pathways. Their mechanisms are well documented; however, little information is available on toxicity in humans, except for high levels of exposure and the carcinogenic effects well established for TRI (group 1, carcinogenic for humans) (Guyton et al. 2014) and TETRA (group 2A, probably carcinogenic for humans). On the contrary, HCBD is not recognized as carcinogenic in humans.

Acknowledgments AVS is supported by the Italian Ministero della Salute (GR-2011-02346985).

Conflict of interest statement The authors declare no conflict of interest.

References

Adams B, Lowpetch K, Thorndycroft F, Whyte SM, Young DW. Stereochemistry of reactions of the inhibitor/substrates L- and D-beta-chloroalanine with beta-mercaptoethanol catalysed by L-aspartate aminotransferase and D-amino acid aminotransferase respectively. Org Biomol Chem. 2005;3:3357–64.

Anders MW. Glutathione-dependent bioactivation of haloalkanes and haloalkenes. Drug Metab Rev. 2004;36:583–94.

Barshteyn N, Elfarra AA. Globin monoadducts and cross-links provide evidence for the presence of S-(1,2-dichlorovinyl)-L-cysteine sulfoxide, chlorothioketene, and 2-chlorothionoacetyl chloride in the circulation in rats administered S-(1,2-dichlorovinyl)-L-cysteine. Chem Res Toxicol. 2009;22:1629–38.

Bartsch H, Malaveille C, Barbin A, Planche G. Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues. Evidence for oxirane formation by P450-linked microsomal mono-oxygenases. Arch Toxicol. 1979;41:249–77.

Bernauer U, Birner G, Dekant W, Henschler D. Biotransformation of trichloroethene: dose-dependent excretion of 2,2,2-trichloro-metabolites and mercapturic acids in rats and humans after inhalation. Arch Toxicol. 1996;70:338–46.

Berndt WO, Mehendale HM. Effects of hexachlorobutadiene (HCBD) on renal function and renal organic ion transport in the rat. Toxicology. 1979;14:55–65.

Birner G, Vamvakas S, Dekant W, Henschler D. Nephrotoxic and genotoxic N-acetyl-S-dichlorovinyl-L-cysteine is a urinary metabolite after occupational 1,1,2-trichloroethene exposure in humans: implications for the risk of trichloroethene exposure. Environ Health Perspect. 1993;99:281–4.

Birner G, Werner M, Ott MM, Dekant W. Sex differences in hexachlorobutadiene biotransformation and nephrotoxicity. Toxicol Appl Pharmacol. 1995;132:203–12.

Birner G, Rutkowska A, Dekant W. N-Acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine and 2,2,2-trichloroethanol: two novel metabolites of tetrachloroethene in humans after occupational exposure. Drug Metab Dispos. 1996;24:41–8.

Birner G, Bernauer U, Werner M, Dekant W. Biotransformation, excretion and nephrotoxicity of haloalkene-derived cysteine S-conjugates. Arch Toxicol. 1997;72:1–8.

Birner G, Werner M, Rosner E, Mehler C, Dekant W. Biotransformation, excretion, and nephrotoxicity of the hexachlorobutadiene metabolite (E)-N-acetyl-S-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine sulfoxide. Chem Res Toxicol. 1998;11:750–7.

Black RM, Read RW. Biological fate of sulphur mustard, 1,1′-thiobis(2-chloroethane): identification of ß-lyase metabolites and hydrolysis products in human urine. Xenobiotica. 1995;25:167–73.

Buben JA, O'Flaherty EJ. Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: a dose–effect study. Toxicol Appl Pharmacol. 1985;78:105–22.

Bull RJ. Mode of action of liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate. Environ Health Perspect. 2000;108 Suppl 2:241–59.

Carrieri M, Magosso D, Piccoli P, Zanetti E, Trevisan A, Bartolucci GB. Acute, nonfatal intoxication with trichloroethylene. Arch Toxicol. 2007;81:529–32.

Cavalleri A, Gobba F, Paltrinieri M, Fantuzzi G, Righi E, Aggazzotti G. Perchloroethylene exposure can induce colour vision loss. Neurosci Lett. 1994;179:162–6.

Cederberg H, Henriksson J, Binderup ML. DNA damage detected by the alkaline comet assay in the liver of mice after oral administration of tetrachloroethylene. Mutagenesis. 2010;25:133–8.

Chiu WA, Ginsberg GL. Development and evaluation of a harmonized physiologically based pharmacokinetic (PBPK) model for perchloroethylene toxicokinetics in mice, rats, and humans. Toxicol Appl Pharmacol. 2011;253:203–34.

Chiusolo A, Defazio R, Casartelli A, Bocchini N, Mongillo M, Zanetti E, et al. Regucalcin down-regulation in rat kidney tissue after treatment with nephrotoxicants. Toxicol Lett. 2008;182:84–90.

Chiusolo A, Defazio R, Zanetti E, Mongillo M, Mori N, Cristofori P, et al. Kidney injury molecule-1 expression in rat proximal tubule after treatment with segment-specific nephrotoxicants:

a tool for early screening of potential kidney toxicity. Toxicol Pathol. 2010;38:338–45.
Cooper AJ, Wang J, Gartner CA, Bruschi SA. Co-purification of mitochondrial HSP70 and mature protein disulfide isomerase with a functional rat kidney high-M(r) cysteine S-conjugate beta-lyase. Biochem Pharmacol. 2001;62:1345–53.
Cooper AJ, Krasnikov BF, Niatsetskaya ZV, Pinto JT, Callery PS, Villar MT, et al. Cysteine S-conjugate β-lyases: important roles in the metabolism of naturally occurring sulfur and selenium-containing compounds, xenobiotics and anticancer agents. Amino Acids. 2011;41:7–27.
Cristofori P, Zanetti E, Fregona D, Piaia A, Trevisan A. Renal proximal tubule segment-specific nephrotoxicity: an overview on biomarkers and histopathology. Toxicol Pathol. 2007;35:270–5.
Cristofori P, Defazio R, Chiusolo A, Mongillo M, Bartolucci GB, Chiara F, et al. Hyaline droplet accumulation in kidney of rats treated with hexachloro-1:3-butadiene: influence of age, dose and time-course. J Appl Toxicol. 2013;33:183–9.
Daoud AH, Irving CC. Methylation of DNA in rat liver and intestine by dimethylnitrosamine and N-methylnitrosourea. Chem Biol Interact. 1977;16:135–43.
Davis SI, Laszlo Pallos L, Wu JQ, Sapp II JH, Cusack C. ATSD R's trichloroethylene subregistry methods and results: 1989–2000. Arch Environ Occup Health. 2005;60:130–9.
Dekant W. Chemical-induced nephrotoxicity mediated by glutathione S-conjugate formation. Toxicol Lett. 2001;124:21–36.
Dekant W. Biosynthesis of toxic glutathione conjugates from halogenated alkenes. Toxicol Lett. 2003;144:49–54.
Dekant W, Vamvakas S. Biotransformation and membrane transport in nephrotoxicity. Crit Rev Toxicol. 1996;26:309–34.
Dekant W, Vamvakas S, Berthold K, Schmidt S, Wild D, Henschler D. Bacterial beta-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene and hexachlorobutadiene. Chem Biol Interact. 1986;60:31–45.
Dekant W, Martens G, Vamvakas S, Metzler M, Henschler D. Bioactivation of tetrachloroethylene. Role of glutathione S-transferase-catalyzed conjugation versus cytochrome P-450-dependent phospholipid alkylation. Drug Metab Dispos. 1987;15:702–9.
Dekant W, Vamvakas S, Koob M, Köchling A, Kanhai W, Müller D, et al. A mechanism of haloalkene-induced renal carcinogenesis. Environ Health Perspect. 1990;88:107–10.
Dekant W, Urban G, Goersmann C, Anders MW. Thioketene formation from α-haloalkenyl 2-nitrophenyl disulfides: models for biological reactive intermediates of cytotoxic S-conjugates. J Am Chem Soc. 1991;113:5120–2.
Dekant W, Vamvakas S, Anders MW. Formation and fate of nephrotoxic and cytotoxic glutathione S-conjugates: cysteine conjugate beta-lyase pathway. Adv Pharmacol. 1994;27:115–62.
Echeverria D, White RF, Sampaio C. A behavioral evaluation of PCE exposure in patients and dry cleaners: a possible relationship between clinical and preclinical effects. J Occup Environ Med. 1995;37:667–80.
Elfarra AA. Halogenated hydrocarbons. In: Goldstein RS, editor. Renal toxicology. New York: Pergamon; 1997. p. 601–16.
Elfarra AA, Anders MW. Renal processing of glutathione conjugates. Role in nephrotoxicity. Biochem Pharmacol. 1984;33:3729–32.
Elfarra AA, Krause RJ. S-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide, a reactive metabolite of S-(1,2,2-Trichlorovinyl)-L-cysteine formed in rat liver and kidney microsomes, is a potent nephrotoxicant. J Pharmacol Exp Ther. 2007;321:1095–101.
Environmental Protection Agency. Toxicological review of trichloroethylene (CAS No. 79-01-6): in support of summary information on the Integrated Risk Information System (IRIS). Washington; 2011.
Gambaro G, Valente ML, Zanetti E, Della Barbera M, Del Prete D, D'Angelo A, et al. Mild tubular damage induces calcium oxalate crystalluria in a model of subtle hyperoxaluria: evidence that a second hit is necessary for renal lithogenesis. J Am Soc Nephrol. 2006;17:2213–9.
Goldsworthy TL, Popp JA. Chlorinated hydrocarbon-induced peroxisomal enzyme activity in relation to species and organ carcinogenicity. Toxicol Appl Pharmacol. 1987;88:225–33.
Green T, Dow J, Foster J. Increased formic acid excretion and the development of kidney toxicity in rats following chronic dosing with trichloroethanol, a major metabolite of trichloroethylene. Toxicology. 2003a;191:109–19.
Green T, Lee R, Farrar D, Hill J. Assessing the health risks following environmental exposure to hexachlorobutadiene. Toxicol Lett. 2003b;138:63–73.
Guengerich FP. Activation of dihaloalkanes by thiol-dependent mechanisms. J Biochem Mol Biol. 2003;36:20–7.
Guha N, Loomis D, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Baan R, Mattock H, Straif K, on behalf of the International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of trichloroethylene, tetrachloroethylene, some other chlorinated solvents, and their metabolites. Lancet Oncol. 2012; 13: 1192–3
Guyton KZ, Hogan KA, Scott CS, Cooper GS, Bale AS, Kopylev L, et al. Human health effects of tetrachloroethylene: key findings and scientific issues. Environ Health Perspect. 2014;122:325–34.
Hibino Y, Wang H, Naito H, Zhao N, Wang D, Jia X, et al. Sex differences in metabolism of trichloroethylene and trichloroethanol in guinea pigs. J Occup Health. 2013;55:443–9.
Hook JB, Ishmael J, Lock EA. Nephrotoxicity of Hexachloro-1:3-butadiene in the rat: the effect of age, sex, and strain. Toxicol Appl Pharmacol. 1983;67:122–31.
International Agency for Research on Cancer. Dry-cleaning, some chlorinated solvents and other industrial chemicals. 63, Lyon; 1995.
International Agency for Research on Cancer. Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. 73, Lyon; 1999.
Irving RM, Elfarra AA. Mutagenicity of the cysteine S-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test. Toxicology. 2013;306:157–61.
Irving RM, Pinkerton ME, Elfarra AA. Characterization of the chemical reactivity and nephrotoxicity of N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine sulfoxide, a potential reactive metabolite of trichloroethylene. Toxicol Appl Pharmacol. 2013;267:1–10.
Ishmael J, Lock EA. Nephrotoxicity of hexachlorobutadiene and its glutathione-derived conjugates. Toxicol Pathol. 1986;14:258–62.
Ishmael J, Pratt I, Lock EA. Necrosis of the pars recta (S3 segment) of the rat kidney produced by hexachloro 1:3 butadiene. J Pathol. 1982;138:99–113.

James EA, Gygi SP, Adams ML, Pierce RH, Fausto N, Aebersold RH, et al. Mitochondrial aconitase modification, functional inhibition, and evidence for a supramolecular complex of the TCA cycle by the renal toxicant S-(1,1,2,2-tetrafluoroethyl)-L-cysteine. Biochemistry. 2002;41:6789–97.

Japan Industrial Safety Association. Carcinogenicity study of tetrachloroethylene by inhalation in rats and mice. Technical Report; 1993.

Johnson PD, Goldberg SJ, Mays MZ, Dawson BV. Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat. Environ Health Perspect. 2003;111:289–92.

Jones TW, Wallin A, Thor H, Gerdes RG, Ormstad K, Orrenius S. The mechanism of pentachlorobutadienyl-glutathione nephrotoxicity studied with isolated rat renal epithelial cells. Arch Biochem Biophys. 1986;251:504–13.

Keil DE, Peden-Adams MM, Wallace S, Ruiz P, Gilkeson GS. Assessment of trichloroethylene (TCE) exposure in murine strains genetically-prone and non-prone to develop autoimmune disease. J Environ Sci Health A Toxic Hazard Subst Environ Eng. 2009;44:443–53.

Kim D, Ghanayem BI. Comparative metabolism and disposition of trichloroethylene in Cyp2e1−/− and wild-type mice. Drug Metab Dispos. 2006;34:2020–7.

Kim HS, Cha SH, Abraham DG, Cooper AJ, Endou H. Intranephron distribution of cysteine S-conjugate beta-lyase activity and its implication for hexachloro-1,3-butadiene-induced nephrotoxicity in rats. Arch Toxicol. 1997;71:131–41.

Kim S, Kim D, Pollack GM, Collins LB, Rusyn I. Pharmacokinetic analysis of trichloroethylene metabolism in male B6C3F1 mice: Formation and disposition of trichloroacetic acid, dichloroacetic acid, S-(1,2-dichlorovinyl)glutathione and S-(1,2-dichlorovinyl)-L-cysteine. Toxicol Appl Pharmacol. 2009;238:90–9.

Klaassen CD, Plaa GL. Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. Toxicol Appl Pharmacol. 1966;9:139–51.

Kline SA, McCoy EC, Rosenkranz HS, Van Duuren BL. Mutagenicity of chloroalkene epoxides in bacterial systems. Mutat Res. 1982;101:115–25.

Kociba RJ, Keyes DG, Jersey GC, Ballard JJ, Dittenber DA, Quast JF, et al. Results of a two year chronic toxicity study with hexachlorobutadiene in rats. Am Ind Hyg Assoc J. 1977;38:589–602.

Koga N, Inskeep PB, Harris TM, Guengerich FP. S-[2-(N7-guanyl)ethyl]glutathione, the major DNA adduct formed from 1,2-dibromoethane. Biochemistry. 1986;25:2192–8.

Krause RJ, Lash LH, Elfarra AA. Human kidney flavin-containing monooxygenases and their potential roles in cysteine s-conjugate metabolism and nephrotoxicity. J Pharmacol Exp Ther. 2003;304:185–91.

Kuo CH, Hook JB. Effects of age and sex on hexachloro-1,3-butadiene toxicity in the Fischer 344 rat. Life Sci. 1983;33:517–23.

Lash LH, Parker JC. Hepatic and renal toxicities associated with perchloroethylene. Pharmacol Rev. 2001;53:177–208.

Lash LH, Sausen PJ, Duescher RJ, Cooley AJ, Elfarra AA. Roles of cysteine conjugate beta-lyase and S-oxidase in nephrotoxicity: studies with S-(1,2-dichlorovinyl)-L-cysteine and S-(1,2-dichlorovinyl)-L-cysteine sulfoxide. J Pharmacol Exp Ther. 1994;269:374–83.

Lash LH, Parker JC, Scott CS. Modes of action of trichloroethylene for kidney tumorigenesis. Environ Health Perspect. 2000;108 Suppl 2:225–40.

Lash LH, Qian W, Putt DA, Hueni SE, Elfarra AA, Krause RJ, et al. Renal and hepatic toxicity of trichloroethylene and its glutathione-derived metabolites in rats and mice: sex-, species-, and tissue-dependent differences. J Pharmacol Exp Ther. 2001;297:155–64.

Lash LH, Qian W, Putt DA, Hueni SE, Elfarra AA, Sicuri AR, et al. Renal toxicity of perchloroethylene and S-(1,2,2-trichlorovinyl)glutathione in rats and mice: sex- and species-dependent differences. Toxicol Appl Pharmacol. 2002;179:163–71.

Livesey JC, Anders MW, Langvardt PW, Putzig CL, Reitz RH. Stereochemistry of the glutathione-dependent biotransformation of vicinal-dihaloalkanes to alkenes. Drug Metab Dispos. 1982;10:201–4.

Lock EA, Reed CJ. Trichloroethylene: mechanisms of renal toxicity and renal cancer and relevance to risk assessment. Toxicol Sci. 2006;91:313–31.

Lock EA, Ishmael J, Hook JB. Nephrotoxicity of hexachloro-1,3-butadiene in the mouse: the effect of age, sex, strain, monooxygenase modifiers, and the role of glutathione. Toxicol Appl Pharmacol. 1984;72:484–94.

Maguire DP, Turton JA, Scudamore CL, Swain AJ, McClure FJ, Smyth R, et al. Correlation of histopathology, urinary biomarkers, and gene expression responses following hexachloro-1:3-butadiene-induced acute nephrotoxicity in male Hanover Wistar rats: a 28-day time course study. Toxicol Pathol. 2013;41:779–94.

Nakajima T, Wang RS, Murayama N, Sato A. Three forms of trichloroethylene-metabolizing enzymes in rat liver induced by ethanol, phenobarbital, and 3-methylcholanthrene. Toxicol Appl Pharmacol. 1990;102:546–52.

National Toxicology Programme. Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) in F344/N rats and B6C3F1 mice (inhalation studies). US DHHS Technical Report No. 311. US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program, Research Triangle Park, NC; 1986.

Nelson SD, Pearson PG. Covalent and noncovalent interactions in acute lethal cell injury caused by chemicals. Annu Rev Pharmacol Toxicol. 1990;30:169–95.

Pähler A, Birner G, Ott MM, Dekant W. Binding of hexachlorobutadiene to alpha 2u-globulin and its role in nephrotoxicity in rats. Toxicol Appl Pharmacol. 1997;147:372–80.

Peden-Adams MM, Eudaly JG, Heesemann LM, Smythe J, Miller J, Gilkeson GS, et al. Developmental immunotoxicity of trichloroethylene (TCE): studies in B6C3F1 mice. J Environ Sci Health A Toxic Hazard Subst Environ Eng. 2006;41:249–71.

Rannug U, Sundvall A, Ramel C. The mutagenic effect of 1,2-dichloroethane on *Salmonella typhimurium* I. Activation through conjugation with glutathione in vitro. Chem Biol Interact. 1978;20:1–16.

Ripp SL, Itagaki K, Philpot RM, Elfarra AA. Species and sex differences in expression of flavin-containing monooxygenase form 3 in liver and kidney microsomes. Drug Metab Dispos. 1999;27:46–52.

Rusyn I, Chiu WA, Lash LH, Kromhout H, Hansen J, Guyton KZ. Trichloroethylene: mechanistic, epidemiologic and other supporting evidence of carcinogenic hazard. Pharmacol Ther. 2014;141:55–68.

Sadeghnia HR, Yousefsani BS, Rashidfar M, Boroushaki MT, Asadpour E, Ghorbani A. Protective effect of rutin on hexachlorobutadiene-induced nephrotoxicity. Ren Fail. 2013;35:1151–5.

Simmons JE, Boyes WK, Bushnell PJ, Raymer JH, Limsakun T, McDonald A, et al. A physiologically based pharmacokinetic model for trichloroethylene in the male Long–Evans rat. Toxicol Sci. 2002;69:3–15.

Tabrez S, Ahmad M. Genotoxicity of trichloroethylene in the natural milieu. Int J Hyg Environ Health. 2012;215:333–8.

Tateishi M, Suzuki S, Shimizu H. Cysteine conjugate beta-lyase in rat liver. A novel enzyme catalyzing formation of thiol-containing metabolites of drugs. J Biol Chem. 1978a;253:8854–9.

Tateishi M, Suzuki S, Shimizu H. The metabolism of bromazepam in the rat-identification of mercapturic acid and its conversion in vitro to methylthio-bromazepam. Biochem Pharmacol. 1978b;27:809–10.

Terracini B, Parker VH. A pathological study on the toxicity of s-dichlorovinyl-l-cysteine. Food Cosmet Toxicol. 1965;3:67–74.

Trevisan A, Cristofori P, Fanelli G, Bicciato F, Stocco E. Glutamine transaminase K intranephron localization in rats determined by urinary excretion after treatment with segment-specific nephrotoxicants. Arch Toxicol. 1998;72:531–5.

Trevisan A, Cristofori P, Fanelli G. Glutamine synthetase activity in rat urine as sensitive marker to detect S3 segment-specific injury of proximal tubule induced by xenobiotics. Arch Toxicol. 1999;73:255–62.

Trevisan A, Maccà I, Rui F, Carrieri M, Battista Bartolucci G, Manno M. Kidney and liver biomarkers in female dry-cleaning workers exposed to perchloroethylene. Biomarkers. 2000;5:399–409.

Trevisan A, Giraldo M, Borella M, Bottegal S, Fabrello A. Tubular segment-specific biomarkers of nephrotoxicity in the rat. Toxicol Lett. 2001;124:113–20.

Trevisan A, Cristofori P, Beggio M, Venturini MB, Di Marco L, Zanetti E. Segmentary effects on the renal proximal tubule due to hexachloro-1,3-butadiene in rats: biomarkers related to gender. J Appl Toxicol. 2005;25:13–9.

Vamvakas S, Dekant W, Berthold K, Schmidt S, Wild D, Henschler D. Enzymatic transformation of mercapturic acids derived from halogenated alkenes to reactive and mutagenic intermediates. Biochem Pharmacol. 1987;36:2741–8.

Vamvakas S, Herkenhoff M, Dekant W, Henschler D. Mutagenicity of tetrachloroethene in the Ames test—metabolic activation by conjugation with glutathione. J Biochem Toxicol. 1989;4:21–7.

Vlaanderen J, Straif K, Ruder A, Blair A, Hansen J, Lynge E, et al. Tetrachloroethylene exposure and bladder cancer risk: a meta-analysis of dry-cleaning-worker studies. Environ Health Perspect. 2014;122:661–6.

Werner M, Birner G, Dekant W. The role of cytochrome P4503A1/2 in the sex-specific sulfoxidation of the hexachlorobutadiene metabolite, N-acetyl-S-(pentachlorobutadienyl)-L-cysteine in rats. Drug Metab Dispos. 1995;23:861–8.

Werner M, Birner G, Dekant W. Sulfoxidation of mercapturic acids derived from tri- and tetrachloroethene by cytochromes P450 3A: a bioactivation reaction in addition to deacetylation and cysteine conjugate beta-lyase mediated cleavage. Chem Res Toxicol. 1996;9:41–9.

World Health Organization. Hexachlorobutadiene (Environmental Health Criteria 156). Geneve: International Programme on Chemical Safety; 1994.

Zanetti E, Chiusolo A, Defazio R, Casartelli A, Cappelletti E, Bocchini N, et al. Evaluation of aging influence on renal toxicity caused by segment-specific nephrotoxicants of the proximal tubule in rat. J Appl Toxicol. 2010;30:142–50.

Zhou YC, Waxman DJ. Activation of peroxisome proliferator-activated receptors by chlorinated hydrocarbons and endogenous steroids. Environ Health Perspect. 1998;106 Suppl 4:983–8.