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Trichloroethylene Biotransformation and its Role in Mutagenicity, Carcinogenicity and Target Organ Toxicity

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Abstract

Metabolism is critical for the mutagenicity, carcinogenicity, and other adverse health effects of trichloroethylene (TCE). Despite the relatively small size and simple chemical structure of TCE, its metabolism is quite complex, yielding multiple intermediates and end-products. Experimental animal and human data indicate that TCE metabolism occurs through two major pathways: cytochrome P450 (CYP)-dependent oxidation and glutathione (GSH) conjugation catalyzed by GSH *S*-transferases (GSTs). Herein we review recent data characterizing TCE processing and flux through these pathways. We describe the catalytic enzymes, their regulation and tissue localization, as well as the evidence for transport and inter-organ processing of metabolites. We address the chemical reactivity of TCE metabolites, highlighting data on mutagenicity of these end-products. Identification in urine of key metabolites, particularly trichloroacetate (TCA), dichloroacetate (DCA), trichloroethanol and its glucuronide (TCOH and TCOG), and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC), in exposed humans and other species (mostly rats and mice) demonstrates function of the two metabolic pathways *in vivo*. The CYP pathway primarily yields chemically stable end-products. However, the GST pathway conjugate *S*-(1,2-dichlorovinyl)glutathione (DCVG) is further processed to multiple highly reactive species that are known to be mutagenic, especially in kidney where *in situ* metabolism occurs. TCE metabolism is highly variable across sexes, species, tissues and individuals. Genetic polymorphisms in several of

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the key enzymes metabolizing TCE and its intermediates contribute to variability in metabolic profiles and rates. In all, the evidence characterizing the complex metabolism of TCE can inform predictions of adverse responses including mutagenesis, carcinogenesis, and acute and chronic organ-specific toxicity.

Keywords

Cytochrome P450-dependent oxidation; glutathione conjugation; kidney; liver; reactive intermediates; trichloroethylene

1. Introduction: Summary of trichloroethylene (TCE) metabolism

TCE is a widespread environmental contaminant that has recently been classified as a human carcinogen by the International Agency for Research on Cancer (IARC) [1] and the U.S. Environmental Protection Agency (U.S. EPA) [2, 3]. TCE metabolism plays a critical role in eliciting its mutagenicity, carcinogenicity and other adverse health effects. Indeed, with the exception of non-specific solvent effects on the neurological system, most organ-specific toxicity has been attributed to specific TCE metabolites. TCE metabolism occurs through two major pathways in humans and laboratory animals, cytochrome P450 (CYP)-dependent oxidation and glutathione (GSH) conjugation [2-5]. Quantitatively, flux through the CYP-dependent oxidation pathway far exceeds that through the GSH conjugation pathway in all species studied, including humans. Metabolites generated by the CYP-dependent oxidation pathway are mostly chemically stable. However, the GSH conjugation pathway generates several highly reactive metabolites that are known to be mutagenic. Due to this difference in the chemical reactivity of the resulting metabolites, interpretations regarding toxicological importance based on quantitative differences in estimated flux of TCE metabolism through these two pathways must be made with caution.

TCE metabolism to mutagenic and toxic moieties remains a significant consideration for predicting and interpreting carcinogenic and other adverse responses to TCE. Additionally, key urinary metabolites from these pathways have utility for estimating exposure in environmental or occupational settings. For example, the American Conference of Governmental and Industrial Hygienists (ACGIH) recommends the use of urinary trichloroacetate (TCA) levels at the end of the work week as a metric for assessing occupational TCE exposure [6]; they recommend a limit of 100 mg TCA/l.

While the basic outlines of the two TCE metabolism pathways depicted in Figs. 1 and 2 have been known for many years [7], additional characterization of metabolites in the past decade has greatly expanded the understanding of TCE metabolism. The sections that follow emphasize recently described metabolites and briefly summarize the major pathways of TCE metabolism, the resulting metabolites, and the enzymes involved. Information on the genotoxic potential of TCE metabolites is highlighted. Inter-organ distribution of TCE metabolites as depicted in Fig. 3 is also discussed. Important sources of variability in TCE metabolism, both within and across species, are identified. The chemical nature and reactivity of TCE metabolites are important determinants of target organ mutagenicity and

tissue, sex, species, and individual differences in catalytic enzyme activity influence the resulting metabolic profiles and rates.

2. CYP-dependent oxidation of TCE

The overall scheme of TCE metabolism through CYP-dependent oxidation is shown in Fig. 1. Several of the resulting metabolites are chemically stable and have been detected in urine, as highlighted in Fig. 1. A summary of the major oxidative metabolites formed from TCE, their site of formation, the species (experimental animals, humans or both) in which they have been identified, and their systemic availability is included in Table 1 (top section). TCE metabolism by the oxidative pathway occurs predominantly in the liver [7]. Other tissues including the lungs [8-10], kidneys [11], and male reproductive organs [12, 13] are also sites of CYP-mediated TCE metabolism. Chemical stability of the metabolite is an important determinant of systemic availability and fate. Relatively stable TCE metabolites may be transported from their site of formation into the bloodstream and delivered to other potential target organs. Chemically unstable and reactive end-products of TCE metabolism, on the other hand, can largely react with DNA, proteins, and lipids near their site of formation.

2.1. TCE metabolites formed through the CYP-dependent oxidation pathway

The initial step in TCE metabolism is catalyzed by one of several CYP enzymes and yields a chemically-unstable cytochrome P450-bound intermediate (TCE-O-CYP) [14]. A large number of metabolites are formed from TCE-O-CYP through either non-enzymatic rearrangement or specific enzymatic reactions. Specifically, TCE-O-CYP can have one of three fates: (1) conversion to chloral (CHL), which is in equilibrium with chloral hydrate (CH); (2) TCE-epoxide (TCE-O); or (3) *N*-hydroxy-acetyl-aminoethanol. Although Miller and Guengerich [14, 15] initially posited that unbound TCE-O did not form to an appreciable extent, subsequent studies by Cai and Guengerich [16-19] demonstrated that TCE-O is in fact generated. These results are consistent with the studies of Forkert [20] on 1,2-dichloroethylene metabolism in mouse Clara cells, which demonstrated formation of an epoxide by a CYP-dependent reaction.

The majority of the flux in CYP-dependent oxidation of TCE is towards CHL/CH [21] via chlorine migration in an oxygenated TCE-CYP transition state [14]. In fact, CHL/CH is typically the most proximal stable metabolite recovered in incubations of tissues, cells, or microsomes with TCE [22]. CHL/CH undergoes either a reduction by alcohol dehydrogenase (ADH) [23] or CYP [24, 25] to generate trichloroethanol (TCOH). Oxidation of CHL/CH by aldehyde dehydrogenase (ALDH) results in formation of trichloroacetic acid (TCA) [26, 27]. Rapid metabolism of CHL/CH in the liver, where the enzymes that participate in further metabolism are abundant, likely explains its detection in smaller amounts than in other tissues (*e.g.*, lung) [10]. TCA is typically the major metabolite of TCE that is recovered, although TCOH is also a significant metabolite. TCOH can be oxidized by CYPs to yield TCA [25], or can undergo glucuronidation by UDP-glucuronosyltransferases (UGTs) to produce trichloroethanol glucuronide (TCOG) [28]. Enterohepatic recirculation of TCOG/TCOH is also known to occur [29]. Both TCOH and TCOG are recovered in

urine. TCA may also undergo dechlorination to yield dichloroacetic acid (DCA) [30], albeit the relevance of these findings to *in vivo* metabolism has been questioned [31].

As illustrated in Fig. 1, there are several possible sources of DCA, including formation through the TCE-O intermediate. TCE-O spontaneously generates dichloroacetyl chloride (DCAC), a chemically unstable and reactive molecule, or oxalic acid (OA), a stable product that is excreted in urine. DCAC undergoes spontaneous dechlorination to produce DCA [32]. Past controversy and uncertainty about the accuracy of measurements of DCA formation highlight the complexity of the oxidative pathway. Conflicting results in the literature, some showing remarkably high levels of formation of DCA, led Ketcha and colleagues [33] to investigate and identify potential sources of artifacts. They concluded that the presence of strong acid in the assay solution causes dechlorination of TCA to DCA, thus overestimating the amount of DCA actually formed *in vivo*. In addition, kinetic analysis of blood levels of DCA and TCA following oral administration of TCE in the mouse also shows that TCA is not the only source of DCA *in vivo* [34, 35].

DCA has an extremely rapid disposition [36, 37] to glyoxylic, oxalic, and monochloroacetic acids. The major pathway for DCA biotransformation is complete dechlorination to glyoxylate in a reaction catalyzed by glutathione transferase ζ (GSTz) [38]. The glyoxylate is subsequently converted to oxalate, glycine, and CO_2 [39]. DCA can also inactivate GSTz [40], which might lead to increased accumulation of DCA secondary to decreased elimination. Reductive monodehalogenation of DCA to monochloroacetic acid, albeit via an unknown *in vivo* mechanism, is a minor pathway that appears to increase with age in rats [41]. Interestingly, DCA is primarily metabolized by enzymes in the cytoplasm unlike other intermediates in the oxidative metabolism pathway (*i.e.*, CH, TCOH, TCA), which are metabolized by enzymes localized primarily in mitochondria and endoplasmic reticulum [42]. Elimination rates of DCA in rats and mice have been reported to be approximately one order of magnitude higher than those in humans [43]. Nonetheless, the U.S. EPA [2] concluded that these may still be high enough in humans to prevent significant accumulation of DCA under most typical exposure conditions.

A third major metabolite postulated to be formed from TCE-O-CYP or TCE-O intermediates is *N*-hydroxy-acetyl-aminoethanol [7]. Although few studies have investigated this metabolite, *N*-hydroxy-acetyl-aminoethanol was found in urine of rats and mice administered TCE and it was estimated that it is formed in amounts greater than those of DCA [44].

2.2. Evidence for CYP-dependent oxidation of TCE in experimental animals

Considerably more data on oxidative metabolism of TCE are available from experimental animal models than from humans or human tissue. Among experimental animal species, studies in rats and mice are the most numerous. These studies have informed identification of the enzymes involved, potential sources of variability, and factors modulating metabolism. In particular, rodent studies have demonstrated the participation of several CYP enzymes in TCE metabolism, including hepatic CYP2E1, CYP1A1/2, CYP2B1/2, and CYP2C11 [45-48]. Forkert et al. [9] also found mouse recombinant CYP2F2 to be active in TCE metabolism. Among several recombinant CYPs tested, rat CYP2E1 exhibited the

highest catalytic efficiency for metabolism of TCE to CH (V_{max}/K_m = 0.79) whereas that for recombinant mouse CYP2F2 was much lower (V_{max}/K_m = 0.11). Thus, although CYP2E1 is considered the primary CYP enzyme for TCE oxidation, other CYPs may play a role, particularly at higher TCE concentrations or in specific extrahepatic tissues. Studies by Kim and Ghanayem [49] with CYP2E1 knockout mice confirmed the predominance of this specific CYP enzyme in TCE metabolism, but also indicated that other CYPs can catalyze TCE oxidation. Although TCE was metabolized to CH in the CYP2E1 knockout mice, it is difficult from these data to extrapolate to normal animals and quantify the role of other CYPs when CYP2E1 is present.

Studies from several *in vitro* incubations with tissue homogenates clearly illustrate the presence of species-dependent differences in TCE oxidative metabolism. For example, the maximal rate of CYP-dependent oxidative metabolism of TCE is 2- to 4-fold faster in mice than in rats; in humans, the maximal rate of CYP-dependent oxidative metabolism of TCE is 5- to more than 10-fold slower than in rats [37, 50-52]. Maximal rates of TCE oxidative metabolism in rodents also differ between males and females. For example, Lash et al. [53] found higher concentrations of CYP-derived metabolites of TCE (*i.e.*, CH, TCA, TCOH) in livers of males than in females; the opposite pattern, however, was observed in kidneys. These results are consistent with higher maximal rates of CYP-dependent metabolism and overall metabolic clearance of TCE in male rats.

The potential role for variability in CYP activity, due to genetic polymorphisms or drug-drug interactions, in TCE metabolism has received some attention. Variability due to CYP polymorphisms has been suggested to have little impact on total flux of TCE metabolism for exposures below saturation, at which hepatic blood flow is limiting [2, 55]. As noted above, knockout of CYP2E1 in the mouse [49] does alter, but not completely abolish, liver CYP-dependent TCE metabolism. However, CYP2E1 knockout had no effect on TCE toxicity in the lung [8]. Considerable inter-strain variability in formation of TCE metabolites from CYP-mediated oxidation of TCE has been also reported [56]. TCE (2100 mg/kg) was administered by intragastric lavage in corn oil to one hybrid and 14 inbred mouse strains; key metabolites were measured at 2, 8 and 24 h in serum. At the 8-h time point (time of maximal metabolite levels in serum), TCA concentrations varied 4.5-fold (from approximately 400 to 1800 nmol/ml) whereas DCA concentrations varied more than 100-fold (from approximately 0.015 to 2 nmol/ml).

Modulation of metabolic conditions with CYP inducers or inhibitors, or alterations in competing pathways, can significantly influence CYP-dependent metabolism of TCE [57]. For example, CYP2E1 induction by pyridine markedly increased TCE metabolism in both kidney and liver cells while attenuation of CYP activity with either a CYP2E1-specific (diethyldithiocarbamate) or a broad-spectrum CYP (metyrapone) inhibitor decreased CYP-dependent TCE metabolism but enhanced GSH conjugation of TCE. While such modulations of CYP activity may not significantly impact the total metabolic flux, they can alter the balance between oxidation and GSH conjugation, as well as metabolism in extra-hepatic tissues that are not flow-limited. Such impacts may also be even more substantial at higher substrate concentrations, where the V_{max} may become more limiting than hepatic blood flow.

2.3. Evidence for CYP-dependent oxidation of TCE in humans

The major oxidative metabolites of TCE, TCA, and TCOH or TCOG, have been detected in human studies in both blood and urine, providing empirical evidence of *in vivo* TCE oxidation in humans [58-63]. Limited information about CYP enzymes involved and their tissue distribution is available from human studies. CYP2E1 is the major CYP enzyme from human liver microsomes that metabolizes organic solvents, including TCE, vinyl chloride, ethylene dichloride, and others [46]. Besides CYP2E1, the other human CYP enzymes reported to have some activity with TCE as substrate include CYP1A1/1A2, CYP2A6, and CYP3A4. There is some disagreement regarding the role of CYP3A4 as Hissink et al. [64] did not detect measurable metabolism of TCE with purified human CYP3A4. Typical activity of CYP2E1 towards TCE is approximately 2-fold and 200-fold higher than that of CYP1A2 and CYP3A4, respectively [7].

CYP distribution may be one factor in determining species-specific differences in TCE metabolism. Although CYPs are distributed in many extrahepatic tissues, the distribution is not uniform across species in terms of either enzyme expression or activity. For example, while CYP2E1 is highly expressed in human liver and testes [12], it is expressed at very low levels in human kidney [65]. Although previous studies [11, 66, 67] detected neither CYP2E1 expression nor its activity in human kidney, use of a newly developed ELISA method showed human kidney cortex to contain about 15% of the level of CYP2E1 in human liver when normalized to microsomal protein (30-122 pmol CYP2E1/mg microsomal protein in liver vs. 5.0-22 pmol CYP2E1/mg microsomal protein). Moreover, when tissue weight and microsomal protein content are factored in, the total amount and activity of CYP2E1 in human liver is more than 50-fold higher than those in human kidney cortex. This sharply contrasts with the situation in rat kidney, which contains easily detectable levels of CYP2E1 and exhibits a relatively high rate of CYP2E1-dependent metabolism of TCE to CH, TCA, and TCOH [68]. Hence, development of physiologically-based pharmacokinetic (PBPK) models for humans must take such significant species-dependent differences into account. Lipscomb et al. [69] modelled data on TCE oxidative metabolism in human liver microsomes and extrapolated rates in this *in vitro* system to account for total TCE metabolism in the human body. They concluded that the extrapolation modestly underestimated total metabolism and recommended inclusion of extrahepatic CYP-dependent metabolism to increase the predictive value of the modelling approach. Similar conclusions were drawn from a Bayesian PBPK model-based analysis of toxicokinetic data on TCE metabolism in mice, rats and humans [70].

2.4. Mutagenic potential of TCE metabolites formed through CYP-dependent oxidation

The chemical reactivity and instability of TCE-O-CYP, TCE-O and DCAC suggest that adduction reactions with biological targets are likely. Indeed, irreversible binding of TCE metabolites from CYP-dependent oxidation to proteins and nucleic acids has been reported in a number of studies [15, 71, 72]. Additionally, studies of TCE genotoxicity in the liver, where oxidative metabolism would presumably predominate, are informative of the genotoxic potential of CYP-derived metabolites. For instance, Chang et al. [73] failed to detect any DNA single-strand breaks in the livers of rats at 4 h after single doses of 1-10 mmol/kg of TCA, DCA, or monochloroacetate (MCA). However, they did detect small

increases in the amounts of DNA single strand breaks (approximately 7%) at 4 h in the livers of mice given the highest doses of TCA (10 mmol/kg) or DCA (5 or 10 mmol/kg). In contrast, Leavitt et al. [74] reported a 1.3- or 2.3-fold increase in mutant frequency in transgenic B6C3F1 mice harbouring the bacterial *lacI* gene after continuous exposure in the drinking water for 60 weeks to 1.0 or 3.5 g DCA/l, respectively. H-ras codon 61 mutations distinct from those in spontaneous tumors were found in mouse liver tumors following DCA administration in the drinking water (1.0 or 3.5 g/l), whereas those following TCA administration in the drinking water (4.5 g/l) exhibited the same mutational spectra as spontaneous tumors [75]. Mixed results are available for genotoxicity of TCE in mammalian cells *in vitro* [76]. One study in human lymphoblasts reported no DNA strand breaks after treatment with either MCA, DCA, TCA, or any of the corresponding aldehydes [73].

Numerous studies have also directly evaluated potential genotoxicity and mutagenicity of TCE metabolites formed through CYP-dependent oxidation, such as CH/CHL, DCA, TCA and, to a lesser extent, TCOH. Strong evidence is available to suggest that CH/CHL may be genotoxic and mutagenic. The evidence is from both *in vivo* and *in vitro* tests, in mammalian and other experimental systems, including studies with and without metabolic activation [2, 76]. The types of damage reported include mutations, chromosomal aberrations, micronuclei, and cell transformation. Importantly, micronuclei in peripheral blood lymphocytes isolated from infants administered CH crystals mixed in breast milk or formula as a single dose of 50 mg/kg of body weight for sedation were significantly increased, consistent with the conclusion that CH is genotoxic in humans [77].

Evidence for other oxidative metabolites suggests a more limited mutagenic potential. The evidence that DCA may be genotoxic is weak to moderate. In bacterial and fungal test systems, only substitution mutation assays were positive [78]. TCA is generally regarded as not genotoxic. TCA was found to be overwhelmingly negative in bacteria and fungi [79, 80], in cultured human lymphocytes or lymphoblasts [73, 81], or in mammalian *in vitro* studies [82, 83]. Most animal *in vivo* studies found no evidence of genotoxicity of TCA [73, 81, 84, 85]. The mutational profile of mouse liver tumors following TCA administration is no different from those in spontaneous liver tumors in the same strain [75, 86]. TCOH has not been evaluated in most recommended genotoxicity screening assays and thus the data should be interpreted with caution. One study found that TCOH was mutagenic in the presence of metabolic activation [87]; however, other studies were negative [80, 88].

3. GSH conjugation of TCE

TCE metabolism by the GSH conjugation pathway (Fig. 2) is initiated by the action of GSH S-transferase (GST) enzymes. The first step in this pathway is an S_N2 nucleophilic displacement reaction of TCE with GSH, releasing Cl^- ion and *S*-(1,2-dichlorovinyl)glutathione (DCVG) as products. Although this initial GSH conjugation step can occur in many tissues, it occurs primarily in the liver owing to first-pass metabolism and the high content of GSTs. In the human and rat liver, the various GST isoforms can account for as much as 5% of total cytosolic protein [89]. Subsequent metabolism through the GSH conjugation pathway occurs primarily in the kidneys [7]. A summary of the site of formation and systemic availability of the major metabolites from the GSH-conjugation pathway is

presented in Table 1 (bottom section). As noted above, although quantitatively the liver is the primary site of GSH conjugation of TCE in the body, the liver is very efficient at excreting GSH conjugates into either bile or plasma. Subsequently, through enterohepatic and renal-hepatic circulation, generally either the cysteine conjugate DCVC or the mercapturate NAcDCVC is delivered to the kidneys for further metabolism or excretion. Additionally, *in situ* GSH conjugation of TCE can occur within the kidneys themselves, primarily the proximal tubules [90-92], establishing an intra-organ cycle of GSH conjugate transport and metabolism (Fig. 3).

3.1. TCE metabolites formed through the GSH conjugation pathway

DCVG, whether formed within the kidneys or in the liver, is processed predominantly in the kidneys by a sequence of two hydrolytic enzymes on the proximal tubular brush-border membrane, γ-glutamyltransferase (GGT) and cysteinylglycine dipeptidase (DP), to yield the corresponding cysteine conjugate, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) [93]. DCVC was first discovered more than 50 years ago in soybean meal as a by-product of TCE extraction [94]. It was identified as the agent causing nephrotoxicity and aplastic anemia in cows, but only nephrotoxicity in most other species. Lock and colleagues [95] verified that DCVC indeed produced both nephrotoxicity and aplastic anemia in cattle, but further showed that none of the other haloalkenyl cysteine conjugates studied caused aplastic anemia in addition to the characteristic nephrotoxicity.

DCVC can be viewed as a major branch point in this metabolic pathway, as it can have three possible fates [7, 96]. First, DCVC can be *N*-acetylated by the microsomal cysteine conjugate *N*-acetyltransferase (NAT) to form the mercapturate *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC) [97]. Besides excretion into urine, NAcDCVC can be deacetylated within the renal proximal tubular cell by aminoacylase III to re-form DCVC [98-100]. Additionally, mercapturates of several nephrotoxic haloalkenes, including NAcDCVC, are substrates for CYP3A enzymes to yield sulfoxides [101-103]. Thus, although NAcDCVC is considered a stable end-product of TCE metabolism and is recovered in urine, it can undergo additional metabolic transformations that serve to reactivate it. These additional fates of the putative end-product of the GSH conjugation pathway highlight both the complexity of TCE metabolism by this pathway and the potential difficulties in using urinary NAcDCVC as a surrogate measurement for overall flux through the GSH conjugation pathway.

Second, DCVC can be a substrate for cysteine conjugate β-lyase (CCBL) activities to generate the reactive *S*-(1,2-dichlorovinyl)thiol (DCVT). DCVT spontaneously rearranges to form either chlorothioketene (CTK) or chlorothionoacetyl chloride (CTAC) [104, 105]. Both of these species are chemically unstable and reactive and are believed to be responsible for formation of covalent adducts derived from DCVC with nucleic acids [106], proteins [107], and phospholipids [108]. CCBL activity has been detected not only in the kidneys, but in liver and other tissues as well. Only renal, as opposed to extra-renal, CCBL activity is toxicologically important for kidney toxicity because of the tissue localization of plasma membrane transporters and several of the enzymes of the GSH conjugation pathway that determine the distribution of TCE metabolites [109]. The overall β-lyase reaction

mechanism is cleavage of a C-S bond to yield a reactive, thioacylating species. However, subsequent studies [110, 111] showed that the reaction mechanism can occur by either direct β-elimination or transamination with a suitable α-keto acid co-substrate to yield either the thiolate or a propionic acid derivative, respectively. The latter is chemically unstable and rearranges to release the thiolate. According to Cooper and colleagues [112, 113], many distinct mammalian enzymes are known to be capable of catalyzing the CCBL reaction. Some of the CCBL enzymes catalyze both β-elimination and transamination reactions, whereas others can only catalyze the former reaction. The relative importance of each of these activities in DCVC bioactivation, however, is not presently known.

Third, DCVC can be a substrate for the flavin-containing monooxygenase (FMO), yielding a reactive *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS) [114]. The FMOs, like the CYPs, represent a multigene family of enzymes [115]. Both enzyme systems also share several other characteristics, including localization in the endoplasmic reticulum, requirement for NADPH as a reductant, and overall catalysis of a mixed-function oxidation reaction. Differences do exist, however, that make some of the functions of the FMOs rather distinctive. For example, although there are more than 50 individual functional CYP enzymes from > 40 gene families in humans [116], there are only 5 FMO genes in mammals. FMOs catalyze oxidation of sulfur-, selenium-, and nitrogen-containing chemicals [117]. Although FMOs and some CYPs share substrates and catalyze the same overall reactions, FMOs have some distinctive substrates, including cysteine *S*-conjugates of various haloalkenes and haloalkanes.

Because of the reactive nature of the various intermediates from this pathway, only NAcDCVC has been recovered in urine of both experimental animals [62, 118] and humans [62, 63] exposed to TCE or DCVC. Of the two possible bioactivation pathways for DCVC, the CCBL and FMO reactions, the former has received the most attention and is thought to account for most of the bioactivation activity for DCVC [7].

3.2. Evidence for GSH conjugation of TCE in experimental animals

Evidence for function of the GSH-dependent pathway *in vivo* in experimental animals comes from detection of either NAcDCVC in urine, or DCVG and DCVC in blood, serum or target tissues, primarily liver and kidney. Bernauer et al. [62], Commandeur and Vermeulen [119], and Larson and Bull [37] reported NAcDCVC in the urine of rats dosed with TCE. As with the case in humans exposed to TCE [62, 63, 120], the ratio of oxidative metabolites of TCE (primarily TCA and TCOH/TCOG) to NAcDCVC was > 1000:1.

DCVG (up to 75 pmol/g liver) and DCVC (up to 400 pmol/g kidney) were detected in female, but not male, F344 rats administered TCE at up to 15 mmol/kg (1.97 g/kg) body weight by corn oil oral gavage [53]. The absence of detectable DCVG in liver or kidney of male rats is consistent with faster breakdown of DCVG in male rats than in female rats. DCVC, in contrast, was detected at levels of 5 to 9 pmol/g liver in female rats, 10 to 25 pmol/g liver in male rats, 7 to 22 pmol/g kidney in female rats, and was not detectable in kidney tissue from male rats. DCVG was detected in whole blood of both male and female rats at levels ranging from 10 to 60 pmol/ml in males and 25 to 95 pmol/ml in females. DCVC was below the limit of detection in blood.

Rusyn and colleagues [34, 35] detected both DCVG and DCVC in serum at levels as high as ~ 40 pmol/ml and 2.4 pmol/ml, respectively, in two studies of male B6C3F1 mice administered 2.1 g TCE/kg body weight by oral gavage. The amounts of serum DCVG in mice were very similar to those reported by Lash et al. [53] in rats. The ability of Kim et al. [34, 35] to detect DCVC at low levels, < 10% of those of DCVG, was achieved through the development of new, highly sensitive detection methods. The studies in rats and in mice provide clear *in vivo* evidence of function of the GSH-conjugation pathway for TCE metabolism. A follow-up study in a panel of 15 inbred mouse strains showed that DCVG levels in serum vary among strains and that DCVG is more abundant than DCVC [56]. Serum DCVG levels were below the limit of detection in 4 strains and varied between 2 and 40 pmol/ml in the other 11 strains. For DCVC, serum concentrations of this penultimate nephrotoxic and nephrocarcinogenic metabolite were below the limit of detection in 6 strains and varied between 1.5 and 3 pmol/ml in other strains.

While occurring in many tissues, the initial step in the GSH conjugation pathway, catalyzed by GST, is most rapid in liver. Lash et al. [92] compared rates of DCVG formation in incubations of 1 or 2 mM TCE with 5 mM GSH in suspensions of isolated hepatocytes and isolated renal proximal tubular cells from male F344 rats. Rates were about 20-fold higher in hepatocytes (5 to 10 nmol DCVG formed/10^6 cells per 60 minutes) than in kidney cells (0.3 to 0.5 nmol DCVG formed/10^6 cells per 60 minutes). Rates of GSH conjugation of TCE were also analyzed in liver and kidney microsomes and cytoplasm from male and female F344 rats and B6C3F1 mice to identify species-, sex-, and tissue-dependent differences. The rate of GSH conjugation of TCE was found to be markedly higher in mouse compared with rat tissues, modestly higher in male compared with female tissues and subcellular fractions, and markedly higher (5- to 25-fold) in liver than in kidney in the corresponding species and sex.

Several families of GST isoenzymes are expressed in mammalian tissues and little information is available concerning substrate specificity of each isoenzyme in general or with regard to TCE. Cummings et al. [121] investigated the GST isoform specificity towards TCE in renal cortical cytoplasm of male F344 rats. Using polyclonal antibodies specific to each isoform, they found that of the three classes of GSTs, only GSTα was detectable. Hence, only activity of the three GSTα dimers, GSTα1-1, GSTα1-2, and GSTα2-2, were assessed using GSTα isoform-selective inhibitors in isolated renal proximal tubular cells and purified GSTα1-2 isoforms. While all three specific GSTα enzymes exhibited similar V_{max} towards TCE (75-150 pmol DCVG formed/min per mg protein), GSTα2-2 had a 50- to 100-fold higher affinity (K_m = 0.074 mM TCE with 5 mM GSH as co-substrate) than GSTα1-1 or GSTα1-2 (K_m = 3.99 and 7.51 mM, respectively). Studies with selective GST isoform inhibitors in renal proximal tubular cells, however, suggested that GSTα1-1 is the primary isoform that is functional in GSH conjugation of TCE in the intact rat kidney cell. These results contrast with those of Hissink et al. [64], who assessed the potential role of different GST isoforms in TCE metabolism by characterizing activity with several purified rat and human GSTs. They reported no activity for GSTα (GSTA) isoforms and significant activity with both GSTμ (GSTM) and GSTπ (GSTP) isoforms. Interestingly, these investigators measured DCVG formation at TCE concentrations ranging from 55 μM to 1 mM but

observed no saturation and reported activity of rat GSTμ3-3 with 0.25 mM TCE as substrate of only ~ 15 pmol/min per mg protein. Thus, it remains unclear which specific GST isoform is responsible for GSH conjugation of TCE.

DCVC metabolism by enzymes that possess CCBL activity can occur in either the cytoplasm or mitochondria. Studies of the CCBL-catalyzed reaction *in vitro* in kidney preparations, with DCVC as a substrate, show that in the rat this reaction is 3- to as much as 10-fold higher than that in analogous human kidney models [97, 110, 111, 122-128]. Rates of CCBL-dependent metabolism of DCVC in rats are considerably slower than those for the initial GSH conjugation step that yields DCVG, suggesting that the CCBL-mediated bioactivation reaction is rate-limiting for the conversion of TCE to nephrotoxic and/or mutagenic metabolites. Although DCVC-induced nephrotoxicity has been studied in mice, metabolic rates for CCBL for that species are not available in the literature.

The precise function of each of the various CCBLs in rat kidney in DCVC bioactivation is unclear. For example, although many studies have focused on CCBL/glutamine transaminase K (GTK) in renal cytoplasm as the enzyme responsible for CCBL-dependent metabolism of DCVC in that subcellular fraction [111, 125, 126, 128, 129], Cooper et al. [130] suggested that a high-molecular-weight enzyme (MW = 330 kDa) is actually responsible for most of the observed metabolic activity in the renal cytoplasm. There are also questions about the precise suborganellar localization of renal mitochondrial CCBL activity [126, 127] and the functional importance of several mitochondrial proteins possessing CCBL activity [126, 127, 131-135].

The other major enzyme system responsible for bioactivation of DCVC is the FMO system. Elfarra and colleagues [136-139] characterized the activity of what was initially detected as a cysteine conjugate S-oxidase and later determined to be a catalytic function of FMOs. Although several FMOs are expressed in rat kidney, FMO3 is the only isoform with significant activity for sulfoxidation of DCVC [136].

3.3. Evidence for GSH conjugation of TCE in humans

In a case report describing TCE ingestion in a 17 year old male attempting suicide, the mercapturate *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC) was detected in urine, as were prominent CYP-derived metabolites, confirming function of the GSH-conjugation pathway for TCE metabolism in humans [90]. Quantitatively, the amount of recoverable NAcDCVC in urine of occupationally exposed workers is several orders of magnitude lower than that of TCA, the major urinary metabolite derived from the oxidative pathway [62, 63]. As noted above, because metabolites of the GSH-conjugation pathway are predominantly chemically reactive and unstable, measurement of NAcDCVC in urine indicates that flux through the pathway has occurred but does not provide an accurate quantitation of that flux. Goeptar et al. [122], Green et al. [97], and Bloemen et al. [140] used the higher ratio of TCA to NAcDCVC in urine and higher measured rates of CYP- versus GST-dependent metabolism in human liver samples to support conclusions that the GSH-conjugation pathway is a minor pathway in humans and that it is not toxicologically important. This conclusion has been challenged because of the consideration discussed above of the reactive

nature of metabolites derived from the GSH-conjugation pathway, and given other data on human metabolism of TCE as discussed below.

Besides detection of the mercapturate in urine as an indicator of exposure to TCE, DCVG was detected in the blood of human volunteers exposed by inhalation to either 50 ppm or 100 ppm TCE for 4 hours [120]. Additionally, peak levels of and the area-under-the-curve (AUC) for DCVG were approximately 2-fold higher in males than in females, suggesting that males experience a higher exposure to reactive metabolite at a given dose of TCE and may be more susceptible to injury. Detection of this key intermediate in blood is consistent with what is known about the handling of GSH and GSH *S*-conjugates among tissues, a process referred to as “inter-organ metabolism” [141, 142] (Fig. 3).

There has been disagreement in the literature about the activity of GSH conjugation of TCE in human and rodent liver and kidney. Lash et al. [143] performed detailed kinetic analyses of GSH conjugation of TCE for human liver and kidney subcellular fractions in isolated hepatocytes and hepatic and renal cytoplasm and microsomes from humans. They reported rates and kinetics of DCVG formation that were generally in agreement with those reported for similar substrates by others. In contrast, Green et al. [97] reported rates of DCVG formation in human liver cytoplasm *in vitro* to be four orders of magnitude lower but did not perform detailed analyses of kinetics. Potential explanations for the differences relating to incubation conditions and analytical methods have been posited, but the controversy remains unresolved.

Four major GST isoforms are expressed in humans, GSTA, GSTM, GSTP, and GSTT [144-148]. Little information is available about the isoform specificity towards TCE, although Hissink et al. [64] reported similar values for purified human GSTM1-1 and GSTP1-1 and no activity with either purified human GSTA1-1, GSTA2-2, or GSTT1-1. Cummings et al. [66] reported that GSTA, GSTP, and GSTT, but not GSTM, proteins are all expressed in freshly isolated human proximal tubular cells. Interestingly, although only a limited number of human kidney samples were tested, both GSTA and GSTP protein expression exhibited a wide variation, consistent with the existence of genetic polymorphisms. In contrast, GSTT protein expression levels were nearly identical in all the samples. It is important, however, to determine if similar variations also exist in GST isoform expression in human liver as the liver is quantitatively the primary site of GSH conjugation of TCE.

Metabolism of DCVG to DCVC occurs in two steps, neither of which is rate-limiting for the overall flux through the GSH-conjugation pathway. Although humans have significantly lower GGT activity than rodents [149], GGT and DP activity on the brush-border plasma membrane of the proximal tubule of human kidney still far exceed that needed to metabolize even very high doses of DCVG. Thus, it is activity of the three enzymes that may subsequently act on DCVC, the CCBL, FMO, or NAT that determines overall flux.

Lash et al. [123] purified CCBL activity from human kidney cytoplasm and found that the intrinsic activity of the human cytoplasmic GTK protein was only about 10% of that from rat kidney cortex cytoplasm. Similarly, McGoldrick et al. [124] measured CCBL activity in

primary cultures of proximal tubular cells from both rat and human kidney, and found activity in rat kidney cells to be threefold higher than that in human kidney cells. Studies in human kidney cytoplasm showed that CCBL activity towards DCVC occurred at a similar rate compared with other typical substrates [150] and that with a preparation of purified GTK from human kidney cytoplasm, was a modest substrate, exhibiting a nearly 3-fold higher rate than substrates like β-chloro-D,L-alanine and Se-methyl-L-selenocysteine but only about 35% of that of *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine [125]. Additionally, although transamination substrates like L-Phe, L-Gln, L-Met, or L-Leu exhibited 2- to 6-fold higher activity than transamination with DCVC, the rate of β-elimination with DCVC as substrate was much higher. Even though expression of CCBL/GTK was found in human kidneys [151], little information is available about the regulation or genetics of this enzyme or any of the other enzymes that may function as CCBLs. In a limited number of human kidney samples, however, McCarthy et al. [150] reported nearly fivefold inter-individual variation in rates of pyruvate formation, suggesting the possibility that genetic polymorphisms may also exist for the CCBL. Because of the function of CCBL activity in generating reactive, sulfur-containing metabolites from DCVC, this finding also suggests that such polymorphisms will be associated with differential mutagenic potentials.

Although metabolism by CCBL has received the most focus in studies of DCVC bioactivation, it may not be the most important pathway by which this occurs, at least in human kidney. Studies of DCVC metabolism and acute cytotoxicity in freshly isolated human proximal tubular cells [11] found little protection from toxicity by preincubation with the CCBL/GTK inhibitor aminooxyacetic acid (AOAA). This contrasts sharply with the situation in rat proximal tubular cells, where DCVC-induced cytotoxicity is almost completely prevented by pre-incubation with AOAA [126].

The alternate explanation for DCVC-induced cytotoxicity in human kidney cells is that FMO may play a more prominent role relative to CCBL whereas the reverse is likely true in rat kidney cells. While human kidney expresses FMO1, FMO3, and FMO5, the FMO1 isoform is the most highly expressed of the three and DCVC is only a substrate for FMO3 [152]. In fact, FMO1 expression averaged about 10-fold higher and FMO5 about 2.5- to 5-fold higher than that of FMO3. Although the sulfoxide metabolite generated by the FMO reaction is extremely reactive [138], more study is needed to fully appreciate the role of this pathway in DCVC metabolism and toxicity in human kidney.

3.4. Mutagenic potential of TCE metabolites formed through GSH conjugation

TCE metabolism through GSH conjugation results in formation of several intermediates with mutagenic potential due to their instability and high reactivity. These include DCVT, CTK and CTAC (Fig. 2). Most of the experimental studies of genotoxicity and mutagenicity of GSH conjugation metabolites of TCE have been performed with DCVG, DCVC, DCVCS and NAcDCVC. Because traditional bacterial mutagenesis assays use liver-derived S9 fraction to test bioactivation, data obtained from such studies are less informative than experimental evidence obtained with kidney homogenates or purified enzymes responsible for biotransformation of TCE to GSH conjugation-derived reactive electrophiles.

One published report examined the mutagenicity of DCVG in *S. typhimurium* strain TA2638 using kidney subcellular fractions for metabolic activation and the β-lyase inhibitor AOAA to inhibit genotoxicity [153]. DCVG exhibited direct-acting mutagenicity, with kidney mitochondria, cytoplasm, or microsomes enhancing the effects. The CCBL inhibitor diminished, but did not abolish the effects. Liver subcellular fractions did not enhance the mutagenicity of DCVG, consistent with kidney *in situ* metabolism playing a significant role in the genotoxicity of TCE metabolites through the GSH conjugation pathway.

DCVC has been the subject of numerous genotoxicity and mutagenicity studies. It was found to be mutagenic in *S. typhimurium* (strains TA100, TA2638, and TA98) using the Ames assay in the absence of S9 [154], and in *S. typhimurium* strain TA2638 with kidney subcellular fractions for metabolic activation [155]. A recent study confirmed these findings [156]. The genotoxicity of DCVC is further supported by the predominantly positive results in other available *in vitro* and *in vivo* assays. Unscheduled DNA synthesis was observed with DCVC treatment of Syrian Hamster fibroblasts [157] or LLC-PK1 cells [158]. Transformation of LLC-PK1 cells [159] or primary cultures of rat kidney cells [160] grown in the presence of DCVC was also reported. DCVC administered *in vivo*, in isolated perfused kidneys, and in isolated proximal tubules of albino male rabbits led to formation of DNA strand breaks [161]. In all the above studies, pretreatment with the CCBL inhibitor AOAA markedly diminished the mutagenic or genotoxic effect of DCVC, demonstrating the requirement for metabolism. In rats that were exposed orally to a single dose of DCVC (1 or 10 mg/kg), no significant DNA damage in rat kidney proximal tubules was observed at the 16-hour sampling time with either dose, or after 1 mg/kg DCVC at the 2-hour sampling time [162]. A statistically significant increase in percentage tail DNA 2 hours after treatment with 10 mg/kg DCVC was found, despite the small number of animals at each dose and sampling time.

Mutagenicity of NAcDCVC was investigated in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA to inhibit genotoxicity [158]. NAcDCVC exhibited direct-acting mutagenicity in the absence of exogenous metabolic activation, with kidney cytoplasm enhancing the effects and AOAA diminishing, but not abolishing the effects.

DCVCS was found to be weakly mutagenic in *S. typhimurium* strain TA100 in the Ames salmonella mutagenicity assay [156]. DCVCS mutagenic activity was approximately 700-fold lower than that of DCVC.

4. Transport and inter-organ distribution of TCE metabolites

Membrane transport and inter-organ translocation processes are important determinants of delivery of metabolic intermediates to sites of further metabolism or toxicity, and thus influence tissue distribution and handling of TCE metabolites [109, 141, 142]. These processes play a critical role in target organ specificity of TCE-induced adverse effects. Several oxidative metabolites, including TCOH, exhibit complex time-dependent patterns consistent with enterohepatic circulation, with excretion of glucuronidated TCOH in bile, regeneration of free TCOH in the small intestine, and reabsorption of TCOH from the gut to

the liver [7, 54]. Metabolites derived from GSH conjugation are also thought to undergo transport and transformation across multiple organs, including the liver, kidneys, and small intestine (Fig. 3).

Although GSH conjugation of TCE occurs predominantly in the liver, it can also occur in the kidneys. Hepatic DCVG is efficiently exported into either bile or plasma by efflux across the canalicular plasma membrane or sinusoidal plasma membrane, respectively. Biliary DCVG is largely degraded by GGT and DP activity to yield DCVC. Through enterohepatic circulation, this DCVC is returned to the liver where it undergoes *N*-acetylation to form the mercapturate NAcDCVC. Hepatic NAcDCVC is then exported to the kidneys for excretion in the urine. Both hepatic DCVG that is exported to plasma and intra-renal DCVG are efficiently translocated to the active site of renal GGT on the proximal tubular brush-border membrane, where it is degraded to DCVC.

A detailed summary of specific carrier proteins and the role of these transporters in GSH metabolism and turnover, susceptibility of the proximal tubules to oxidative and toxicant stresses, and modulation in disease and other pathological processes has been recently published [141]. DCVG that is presented to the kidneys can either undergo glomerular filtration and enter the tubular lumen or be in the renal periplasmic space. The former is metabolized by GGT and DP just as in the biliary tract to generate DCVC, which is transported into the proximal tubular cell by amino acid transporters for further metabolism. Renal plasma DCVG can be a substrate for one of three putative carriers on the basolateral plasma membrane. Work by Lash and Jones [163, 164] defined the processes of basolateral GSH uptake, demonstrating energy-dependent and both Na^+-coupled and Na^+-independent transport. They further showed that, like many membrane transporters, these carriers have somewhat broad substrate specificities and can also transport various γ-glutamyl amino acids and GSH *S*-conjugates, including DCVG [165]. Although substrate specificity and energetics studies proposed the potential function of three basolateral membrane carriers, the sodium dicarboxylate carrier-3 (NaC3; *Slc13a3*) and the organic anion transporter 1 and 3 (OAT1/3; *Slc22a6/8*), definitive evidence has only been obtained for the function of OAT3 [166].

5. Variability in TCE metabolism and TCE metabolite transport

Besides species differences, another important consideration for TCE metabolism in humans is inter-individual variability, with potential influences on both oxidative and GSH conjugation metabolism pathways. Lipscomb and colleagues [167] reported 2- to nearly 4-fold variations in CYP content and a 5-fold range of rates of oxidative metabolism of TCE in human liver microsomes. This was most closely correlated with expression of CYP2E1 protein. Similarly, Elfarra et al. [50] reported a 4-fold variation in the rate of TCE metabolism to CH and TCOH in a limited number of samples of human liver microsomes. In addition to CYPs, particularly CYP2E1, overall metabolism of TCE by the oxidative pathway is also determined by expression and activity of ALDH and ADH (Fig. 1). Hence, variation in expression and/or activity of these enzymes may impact the rate at which TCE is cleared and converted to end-products such as TCOH, TCA, and DCA. The rate at which potentially mutagenic metabolites (i.e., *N*-hydroxy-acetyl-aminoethanol and DCAC) are

formed will also vary according to these factors, presumably leading to variations in toxic outcomes. Major (up to 10-fold) inter-strain variation in both CYP- and GST-mediated metabolism of TCE has also been reported in mice [56]. It is important to note, however, that the limitations on variations in bioactivation imposed by hepatic blood flow may dampen the effects of differences in enzyme expression [55]. Lipscomb and colleagues [55] developed a PBPK model for study of TCE metabolism and demonstrated that hepatic blood flow delivers substrates to the liver at rates that are generally slower than those of the bioactivation reactions, thereby limiting the impact of differences in rates of those reactions.

In humans, three major underlying causes contribute to variation in expression and/or activity of drug metabolism enzymes, such as CYP2E1, ALDH, and ADH. The first cause is the existence of genetic polymorphisms, inherited mutations in genes that result in altered expression levels and/or activity of enzymes in at least 1% of the population. The overall implication of such genetic variation is that individuals with altered levels of a given enzyme may exhibit altered susceptibility to toxic effects of a chemical that is metabolized by that enzyme as compared to the rest of the population who express normal levels of the protein. Cataloguing of such individual variation and modulation of drug levels to account for this in a therapeutic regimen is the basis for the current drive for so-called “personalized medicine.” For toxic chemicals such as TCE, application of these principles to human health risk assessment represents an important refinement whose potential is only recently being realized [168]. Bronley-Delancy et al. [26] investigated oxidative metabolism of TCE in cryopreserved human hepatocytes and found large inter-individual variation in kinetic parameters for TCOH and TCA formation. Cells were incubated with CH and K_m values ranged between 0.001 to 2.44 mM for TCOH formation and 0.005 to 1.27 mM for TCA formation; similarly, V_{max} values ranged between 0.54 and 222 nmol TCOH formed/min per mg protein and 0.14 to 158 nmol TCA formed/min per mg protein. No correlation was seen, however, between V_{max} and ADH or ALDH genotype among the limited number of human liver samples. Polymorphisms in CYP2E1 are also known [169-171]. However, the potential impact of such polymorphisms on TCE metabolism was not investigated.

The second major underlying cause for human variation in drug metabolism enzyme expression and/or activity is the influence of prior or concurrent exposure to a chemical or drug that is a substrate for the enzyme in question. This exposure often results in either inhibition of activity or induction of enzyme expression. For example, ethanol is metabolized by CYP2E1, ADH, and catalase, and chronic exposure to ethanol leads to increased activity of CYP2E1 [172]. Such changes would be expected to increase metabolism of TCE.

The third major underlying cause of human variation in drug metabolism enzyme expression and/or activity is the existence of a disease or other chronic, pathological state. For example, chronic diseases such as diabetes modulate expression and activities of various drug metabolism enzymes and transporter proteins [173].

Genetic polymorphisms have also been identified for OAT1 and OAT3 [174-176]. Thus, different subpopulations of humans may have a markedly different capacity to accumulate DCVG or DCVC, thus altering their basic pharmacokinetics and susceptibility to

nephrotoxicity. A better understanding of the implications of genetic polymorphisms in the genes for membrane transporters is relevant not only to toxicants but to therapeutics as well [177]. Further, expression and function of OATs and other organic anion transporters have been shown to exhibit gender-dependent differences in both humans and experimental animals [178-181], suggesting that transport differences are another contributing factor to gender differences in susceptibility to TCE metabolites. Protein kinase C or other signaling pathways can regulate gene expression or function of several of the organic anion transporters [182-184], adding another dimension to potential factors that can alter target cell accumulation of TCE metabolites.

6. Summary and conclusions

Despite nearly 40 years of research on the metabolism and target-organ toxicities associated with TCE, significant questions remain about how this environmental contaminant is handled in the body and its mechanisms of action in various target tissues. An appreciation of the chemical nature and reactivity of metabolites formed, how the enzymes involved are regulated, and tissue-, sex-, and species-specific differences are critical for understanding human health risks, including acute organ-specific toxicity, mutagenesis, and carcinogenesis. The complexities of TCE metabolism remain a continuing research challenge. Knowledge gaps persist regarding the extent of metabolism variability across target organs, species-, and individuals, including the amounts of reactive metabolites formed and their relative contribution to TCE-induced mutation and cancer.

There is consensus among experts that the CYP-mediated oxidation of TCE is a metabolic pathway that predominates quantitatively in all species studied, especially at environmentally-relevant concentrations or doses of TCE. Most of the TCE metabolites generated by the CYP-mediated oxidation pathway are chemically stable with evidence of systemic availability (Table 1) and urinary excretion (Fig. 1). With the exception of CHL/CH, these oxidative metabolites may contribute to carcinogenicity through mechanisms other than direct interaction with DNA. On the other hand, the high chemical reactivity and instability of metabolites generated by the GST pathway (Table 1 and Fig. 2) strongly argue for direct genotoxicity and mutagenicity as mechanistic events that may lead to cancer. A suggestion that the von-Hippel-Lindau tumor suppressor gene (VHL) mutations in renal cell carcinoma of subjects with occupational exposure to TCE are evidence of genotoxicity of TCE in humans [185, 186] has not been verified, and recent studies have reported *VHL* mutations with high frequency in renal cell carcinoma, irrespective of exposure to TCE and other chlorinated solvents [187, 188]. Still, recent molecular epidemiology data demonstrating elevated kidney cancer risks among GST-active individuals, and attenuation among GST-null individuals [189], strongly supports the conclusion that GSH conjugation of TCE is toxicologically significant, at least for the kidney as a target organ. Furthermore, the influence of these genetic polymorphisms adds an additional degree of complexity and uncertainty to understanding TCE-induced mutagenicity and carcinogenicity.

The quantitative extent of TCE flux through the GSH conjugation pathway remains uncertain. Difficulties in quantifying flux through pathways that generate reactive species

notwithstanding, new sensitive methods to quantify certain TCE metabolites are being developed and should help address remaining uncertainties in both human and experimental animal studies. Equally important is to determine the toxicity and mutagenicity of TCE metabolites from the GSH conjugation pathway *in vivo*. Few data are available from sub-chronic or chronic experimental animal studies to inform adverse health outcomes of DCVG and DCVC.

An additional, important consideration for human health risk assessment is variability in rates of TCE metabolism across species, tissues and individuals. For species differences, rates of both CYP- and GST-dependent flux are faster in rodents than in humans, with those in mice generally being markedly faster than in rats. Knowledge of such species differences in metabolic rates is important in using data from experimental animals to make predictions for humans, although differences in rates may not translate into differences in total flux, due to other physiological factors such as hepatic blood flow. For tissue differences, although the liver is universally the primary site of TCE metabolism amongst all species studied, extrahepatic sites are important for many tissue-specific effects in several species (Fig. 3). For individual variability, genetic polymorphisms exist for CYP2E1, GSTs, CCBL, and FMO, which need to be taken into account when making predictions for humans. Likewise, considerable variability in TCE metabolism has been observed among mouse inbred strains and understanding whether the inter-strain variability in TCE metabolism is associated with the differences in toxicity outcomes may provide important clues as to the quantitative underpinnings of various toxic effects of TCE, including mutagenicity [190]. Finally, the complexities of using epidemiology studies for understanding TCE metabolism and mechanisms of action are illustrated by a recent retrospective cohort study [191].

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Abbreviations

ACGIH	American Conference of Governmental and Industrial Hygienists
ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
AOAA	aminooxyacetic acid
AUC	area-under-the-curve
BBM	brush-border membrane
BLM	basolateral plasma membrane
CCBL	cysteine conjugate β-lyase
CH	chloral hydrate
CHL	chloral

CM	canalicular plasma membrane
CTAC	chlorothionoacetyl chloride
CTK	chlorothioketene
CYP	cytochrome P-450
DCA	dichloroacetate
DCAC	dichloroacetyl chloride
DCVC	*S*-(1,2-dichlorovinyl)-L-cysteine
DCVG	*S*-(1,2-dichlorovinyl)glutathione
DCVS	*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide
DCVT	*S*-(1,2-dichlorovinyl)-thiol
DP	cysteinylglycine dipeptidase
FMO	flavin-containing monooxygenase
GGT	γ-glutamyltransferase
GSH	glutathione
GST	glutathione *S*-transferase
GTK	glutamine transaminase K
EPA	Environmental Protection Agency
IARC	International Agency for Research on Cancer
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MCA	monochloroacetate
NAcDCVC	*N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine
NAT	cysteine conjugate *N*-acetyltransferase
OA	oxalic acid
OAT	organic anion transporter
PBPK	physiologically-based pharmacokinetic
SM	sinusoidal plasma membrane
TCA	trichloroacetate
TCE	trichloroethylene
TCE-O	trichloroethylene epoxide
TCOG	trichloroethanol glucuronide
TCOH	trichloroethanol
UGT	UDP-glucuronosyltransferase

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Research Highlights

- Trichloroethylene is mutagenic and carcinogenic in humans and animals
- Trichloroethylene undergoes both cytochrome P450- and glutathione-dependent metabolism
- Glutathione-derived metabolites of trichloroethylene are mutagenic
- Species-, sex-, and tissue-dependent differences exist in trichloroethylene metabolism

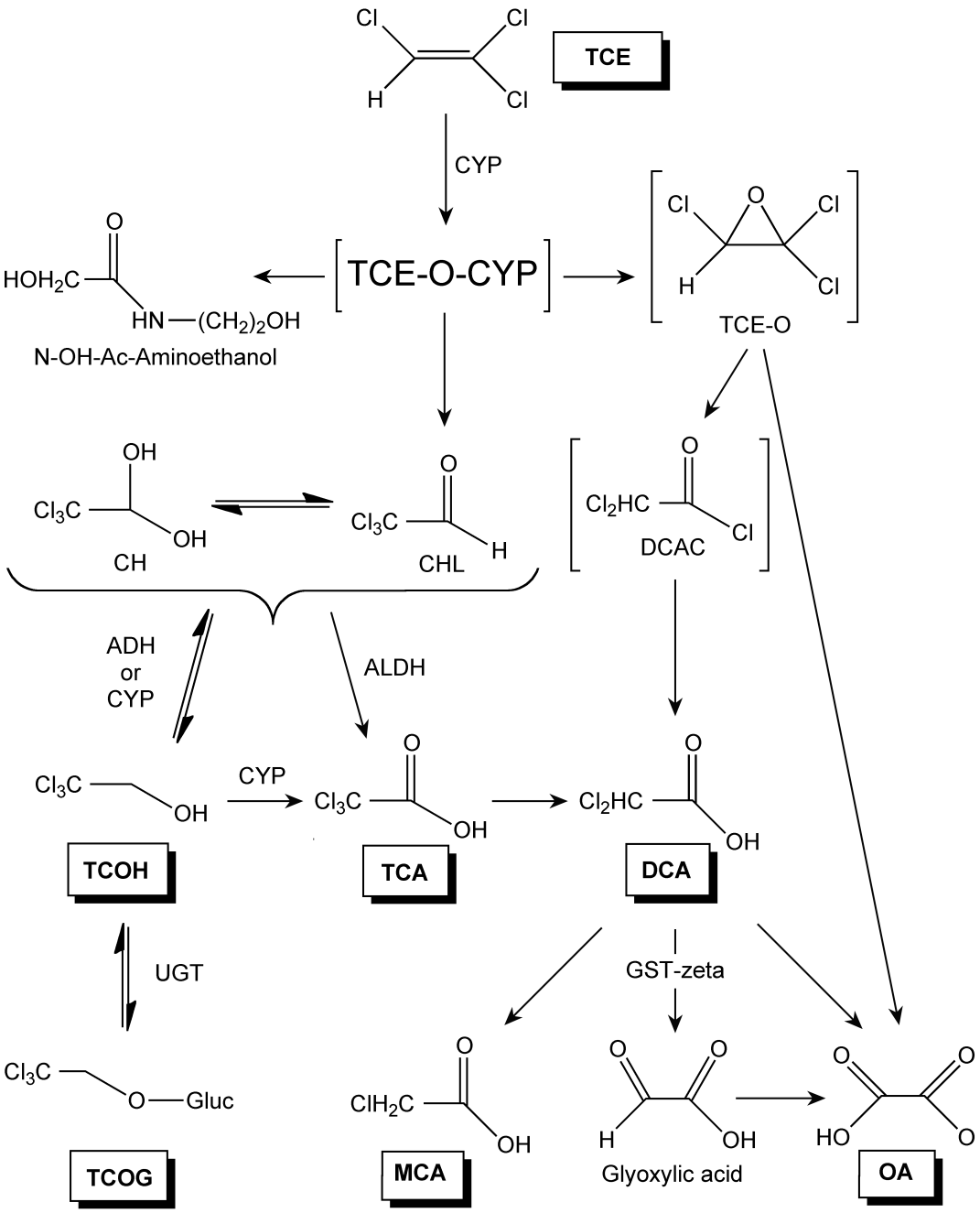

Fig. 1. Scheme for biotransformation of TCE by oxidative metabolism

TCE undergoes cytochrome P450 (CYP)-dependent oxidation to form either a TCE-CYP intermediate or an epoxide intermediate. Further processing through either non-enzymatic rearrangements or actions of aldehyde dehydrogenase (ALDH), alcohol dehydrogenase (ADH), CYPs, or GSH S-transferase zeta (GSTZ) yield a variety of metabolites, including chloral (CHL) and chloral hydrate (CH), dichloroacetate (DCA), trichloroacetate (TCA), trichloroethanol (TCOH) and its glucuronide (TCOG), monochloroacetate (MCA), and oxalate (OA). Names of metabolites that are recovered in urine are shown in boxes and

those that are chemically unstable or reactive are shown in brackets. Other abbreviations: TCE-O, trichloroethylene oxide; DCAC, dichloroacetyl chloride; N-OH-Ac-Aminoethanol, N-hydroxyacetyl aminoethanol; UGT, UDP-glucuronosyltransferase.

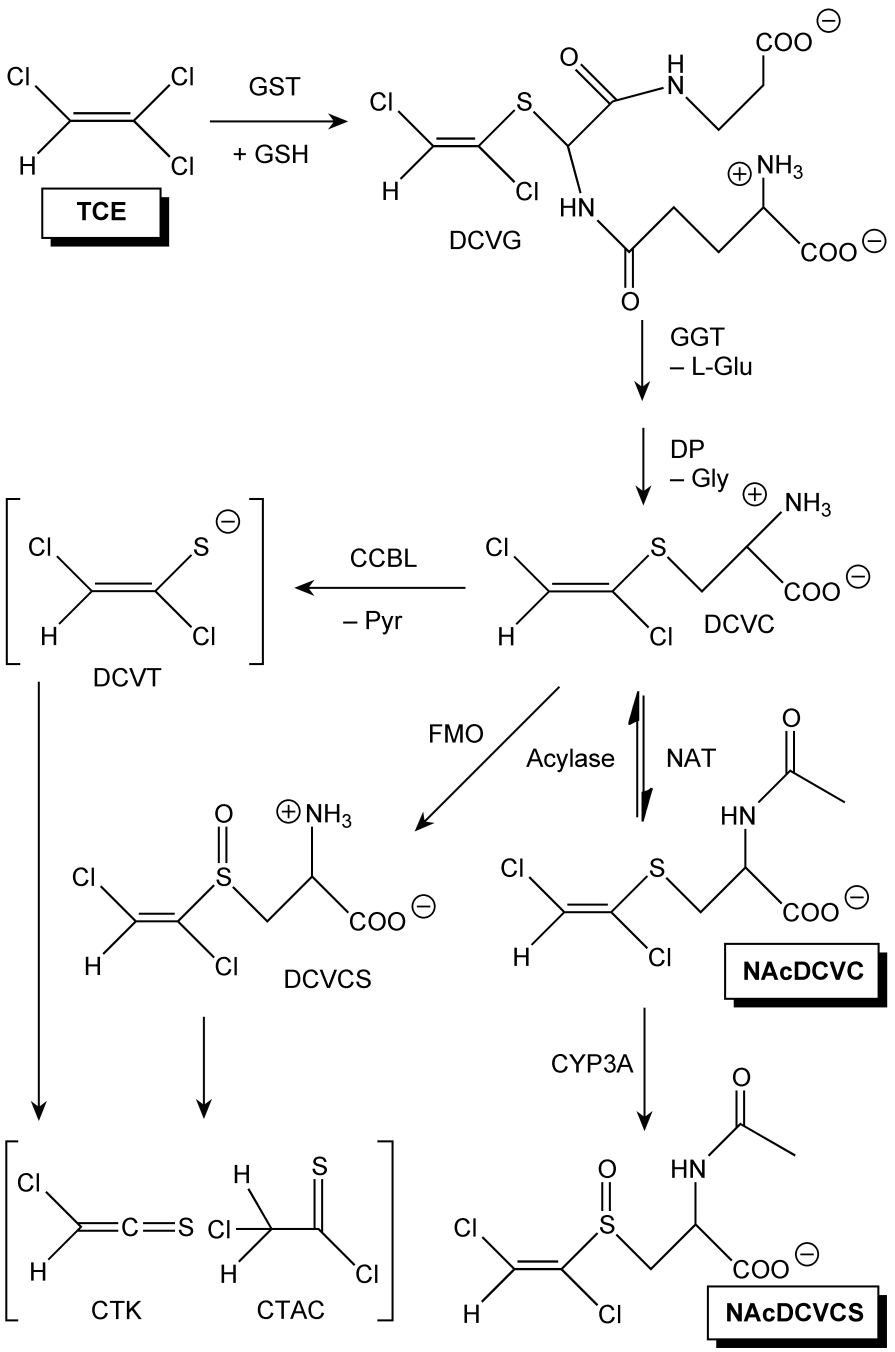

Fig. 2. Scheme for biotransformation of TCE by the GSH conjugation pathway

TCE undergoes conjugation with GSH to yield the GSH S-conjugate DCVG. After processing to yield the cysteine S-conjugate DCVC, three potential fates are detoxication to yield the mercapturate NAcDCVC or bioactivation by either the cysteine conjugate β-lyase to yield dichlorovinylthiol, which rearranges to yield thioacylating species, or the flavin-containing monooxygenase to yield DCVC sulfoxide. The mercapturate can also be deacetylated to regenerate DCVC or it can undergo CYP3A-dependent sulfoxidation. Names of metabolites that are recovered in urine are shown in boxes and those that are chemically

unstable or reactive are shown in brackets. Abbreviations: CYP3A, cytochrome P-450 3A; CTAC, chlorothionoacetyl chloride; CTK, chlorothioketene; DCVC, S-(1,2-dichlorovinyl)-L-cysteine; DCVG, S-(1,2-dichlorovinyl)glutathione; DCVCO, DCVC sulfoxide; DCVT, 1,2-dichlorovinylthiol; DP, dipeptidase; FMO, flavin-containing monooxygenase; GGT, γ-glutamyltransferase; GSH, glutathione; GST, GSH S-transferase; NAcDCVC, N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine; NAcDCVCS, NAcDCVC sulfoxide; NAT, N-acetyltransferase.

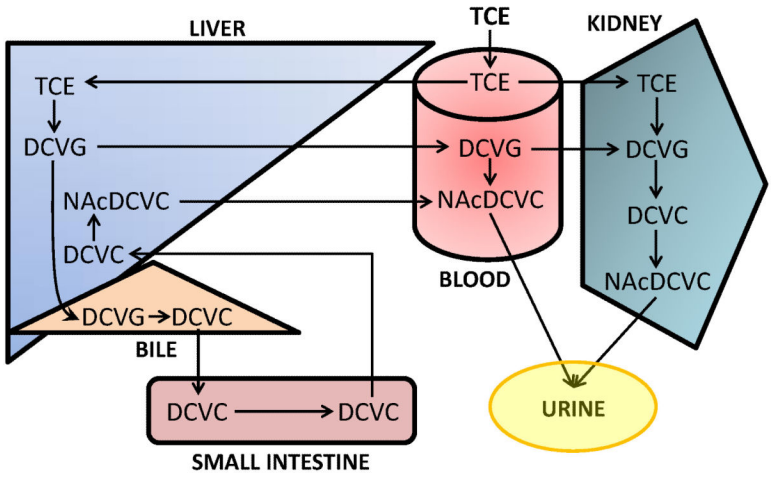

Fig. 3. Inter-organ pathways for metabolism of TCE by the GSH conjugation pathway

The scheme summarizes the tissue localization of metabolic reactions and membrane transporters involved in whole body metabolism of TCE by the GSH conjugation pathway. Most of the DCVG formation occurs in the liver, which is very efficient at excreting it into bile or plasma. Biliary DCVG is processed to yield the cysteine conjugate DCVC, which then returns to the liver by enterohepatic recirculation. Most of the hepatic DCVC is N-acetylated to form the mercapturate NAcDCVC, which is efficiently excreted into plasma. Plasma DCVG and NAcDCVC move through the blood and are extracted by the kidneys. DCVG formation also occurs, although to a lesser extent, within the kidneys themselves.

Table 1

Summary of metabolite formation and systemic availability for TCE

Compound or metabolite	Portal of entry or tissues where formed (Animals/Humans)	Systemic availability (A/H)
TCE	Lung GI Skin	Yes (A, H)
TCE metabolites through CYP-dependent oxidation pathway		
TCE-O **DCAC**	Liver (A, H) Lung (A, H) Testes (A, H)	No
CH/CHL	Liver (A, H) Lung (A, H) Testes (A, H)	Yes
TCOH	Liver (A, H) Lung (A) GI (A, H) Testes (A, H)	Yes
TCA	Liver (A, H) Lung (A, H) Testes (H)	Yes
TCOG	Liver (A, H)	Yes
DCA	Liver (A) Lung (A) Testes (H)	Yes (low amount)
TCE metabolites through GSH conjugation pathway		
DCVG	Liver (A, H) Kidney (A, H)	Yes
DCVC	Liver (A, H) Kidney (A, H)	Yes
DCVCT **DCVCS** **CTK/CTAC**	Kidney (A, H) Hematopoietic (A)	No
NAcDCVC **NAcDCVCS**	Liver (A, H) Kidney (A, H)	Yes