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

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ABSTRACT

Previously, we have provided evidence that cytochromes P450 (P450s) and flavin-containing monoxygenases (FMOs) are involved in the oxidation of S-(1,2,2-trichlorovinyl)-L-cysteine (TCVC) in rabbit liver microsomes to yield the reactive metabolite TCVC sulfoxide (TCVCS). Because TCVC is a known nephrotoxic metabolite of tetrachloroethylene, the nephrotoxic potential of TCVCS in rats and TCVCS formation in rat liver and kidney microsomes were investigated. At 5 mM TCVC, rat liver microsomes formed TCVCS at a rate nearly 5 times higher than rat kidney microsomes. At 1 mM TCVC only the liver activity was detectable. TCVCS formation in liver and kidney microsomes was dependent upon the presence of NADPH and was inhibited by the addition of methimazole or 1-benzylimidazole, but not superoxide dismutase, catalase, KCN, or deferoxamine, consistent with the involvement of both FMOs and P450s. Rats given TCVCS at 230 μmol/kg i.p. exhibited acute tubular necrosis at 2 and 24 h after treatment, and they had elevated blood urea nitrogen levels at 24 h, whereas TCVC was a much less potent nephrotoxicant than TCVCS. Furthermore, pretreatment with aminooxyacetic acid enhanced TCVC toxicity. In addition, reduced nonprotein thiol concentrations in the kidney were decreased by nearly 50% 2 h after TCVCS treatment compared with saline-treated rats, whereas the equimolar dose of TCVC had no effect on kidney nonprotein thiol status. No significant lesions or changes in nonprotein thiol status were observed in liver with either TCVC or TCVCS. Collectively, the results suggest that TCVCS may play a role in TCVC-induced nephrotoxicity.

Tetrachloroethylene (TETRA), a widely used dry cleaning solvent and metal degreaser, is a common surface and groundwater pollutant, air pollutant, and Superfund site contaminant. TETRA is a known rodent liver and kidney toxicant. Long-term exposure studies have also identified TETRA as a liver carcinogen in mice (National Toxicology Program, 1986; Anna et al., 1994) and as a kidney carcinogen in rats (Dekant et al., 1986; National Toxicology Program, 1986; Green et al., 1990). The International Agency for Research on Cancer considers TETRA as “probably carcinogenic to humans (group 2A)” (International Agency for Research on Cancer, 1995). Increases in leukemias and cancers of the bladder, esophagus, and cervix have been observed in workers exposed to TETRA at levels well below the Dutch occupational exposure limit (7.9 versus 240 mg/m3) showed increased levels of retinol-binding protein compared with controls (Verplanke et al., 1999). Collectively, these results indicate that exposure to TETRA may cause renal tubular toxicity in humans.

Whereas TETRA is metabolized by both cytochromes P450 and glutathione S-transferases, only the latter enzymes have been implicated in the formation of nephrotoxic and nephrocarcinogenic metabolites (Dekant et al., 1986, 1987; Lash et al., 1981; Duh and Asal, 1984; Antilla et al., 1995). Studies of workers exposed to low concentrations of TETRA have reported increased urinary levels of lysozyme, albumin, β-glucuronidase, and the brush-border membrane antigens B50, BBA, and HF5 (Franchini et al., 1983; Mutti et al., 1992). Another study of Dutch workers exposed to TETRA at levels well below the Dutch occupational exposure limit (7.9 versus 240 mg/m3) showed increased levels of retinol-binding protein compared with controls (Verplanke et al., 1999). Collectively, these results indicate that exposure to TETRA may cause renal tubular toxicity in humans.
al., 1998; Lash and Parker, 2001). The glutathione conjugation of TETRA occurs primarily in the liver, and it results in the formation of S-(1,2,2-trichlorovinyl)-L-cysteine (TCVG), which has been shown to be more toxic to isolated kidney cells from male rats than TETRA (Lash et al., 2002). TCVG can be translocated to the kidneys via the circulation. In kidney cells, TCVG can be cleaved to the corresponding cysteine S-conjugate, S-(1,2,2-trichlorovinyl)-L-cysteine (TCVC; Fig. 1), by γ-glutamyl transpeptidase (GGTP) and dipeptidase. Hepatic TCVG may also be excreted in bile, and it may be converted to TCVC by enzymes present in biliary epithelial cells and intestinal microflora before it can be reabsorbed and concentrated in kidney cells. The relevance of this metabolic pathway to humans was evidenced by the detection of the mercapturic acid N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine in the urine of humans exposed to TETRA (Birner et al., 1996; Völkel et al., 1998). In this regard, the mercapturic acid of TCVC was presumably formed from TCVC by hepatic and/or renal N-acetyltransferases.

Two additional TCVC metabolic reactions have been characterized. The first results in cleavage of TCVC by cysteine S-conjugate β-lyases (β-lyases), present in kidney cytosol and mitochondria, to produce a sulfur species (Fig. 1) that readily loses chloride ion to form dichlorothioketene. Formation of dichlorothioketene has been implicated in TETRA-induced nephrotoxicity and nephrocarcinogenicity, because of its reactivity toward renal macromolecules (Dekant et al., 1988; Vamvakas et al., 1989a,b; Pähler et al., 1999). In the second metabolic reaction, TCVC is oxidized by flavin-containing monooxygenases (FMOs) or cytochromes P450 to form S-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide (TCVCS) (Ripp et al., 1997). In rabbit liver microsomes, the TCVC S-oxidase activity was dependent on the presence of NADPH, it was stereoselective (diastereomer 2 making up 65–100% of the combined sulfoxide peak area), and it was inhibited by the inclusion of methimazole, an alternative FMO substrate, or 1-benzylimidazole, a generic cytochrome P450 inhibitor. Using cDNA-expressed rabbit FMO isozymes, TCVC has been shown to be a substrate for FMO3, but not for FMO1, FMO2, or FMO5. Because TCVCS reacts readily with glutathione (t1/2 = 20 min) in vitro, but TCVC is unreactive (Ripp et al., 1997), TCVCS formation has been implicated in TCVC-induced nephrotoxicity.

In the current study, we characterized the ability of rat liver and kidney microsomes to metabolize TCVC to TCVCS in vitro. We also investigated TCVCS nephrotoxicity in rats in vivo and determined the effect of aminooxyacetic acid (AOAA), a potent β-lyase inhibitor, on TCVC toxicity. Furthermore, the effects of TCVC and TCVCS on hepatic and renal NPT status were also examined. Collectively, these experiments were designed to clarify the potential roles of S-oxidases and β-lyases in TCVC-induced nephrotoxicity.

Materials and Methods

Materials. TETRA, L-cysteine, AOAA, catalase, superoxide dismutase, deferoxamine, and 5,5′-dithiobis(2-nitrobenzoic acid) were purchased from Sigma-Aldrich (St. Louis, MO). Ktis to determine serum glutamate-pyruvate transaminase (SGPT), serum glutamate-oxalacetate transaminase (SGOT), and urinary GGT activities, and serum and urinary glucose and blood urea nitrogen (BUN) levels were also obtained from Sigma-Aldrich. Methimazole, 1-benzylimidazole, trifluoroacetic acid (TFA), and NaBH₄ were purchased from Aldrich Chemical Co. (Milwaukee, WI). HPLC-grade acetonitrile (ACN) was obtained from EM Scientific (Gibbstown, NJ). TCVC and TCVCS were prepared as described previously (Ripp et al., 1997), and purity was >95% as determined by HPLC. All other chemicals and reagents were of the highest grade commercially available.

Microsomal Preparations and Incubations. Unless otherwise stated, the buffer used in all experiments was 0.1 M KH₂PO₄, 0.1 M KCl, and 5 mM EDTA, pH 7.4. “Washed” microsomes were prepared as described previously (Sausen and Elfarra, 1990) from livers and kidneys of male Sprague-Dawley rats (225–250 g) obtained from Sasco (Omaha, NE). The microsomal protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Microsomal incubations (the final incubation volume was 0.5 ml) typically were carried out as follows: microsomal suspensions (0.3–0.7 mg of protein suspended in the incubation buffer described above) were preincubated in the presence or absence (controls) of 1.5 mM NADPH for 5 min at 37°C in a Dubnoff shaking water bath (Precision Scientific, Chicago, IL). TCVC was dissolved in incubation buffer with the aid of a few drops of 0.1 N NaOH, pH 8.0. TCVC (150 μl) was added to the enzymatic incubation to start the metabolic reaction; the pH of the reaction mixture was approximately pH 7.6 after the addition of TCVC solution (final concentration 5 mM). The reaction was continued for 20 min at 4°C at 3000 rpm to remove precipitated proteins in a Beckman model TJ-6R benchtop centrifuge (Beckman Coulter, Inc., Fullerton, CA). The supernatants were filtered using 0.2-μm Acrodisc filters, and they were analyzed directly by HPLC (described below).

Fig. 1. Possible pathways for the bioactivation of S-(1,2,2-trichlorovinyl)-L-cysteine.
When microsomal incubations were carried out in the presence of enzyme inhibitors, alternative substrates, or other reaction modifiers, these compounds were preincubated with NADPH and microsomes for 5 min before 5 mM TCVC (final concentration) was added. The reaction time was 30 min. The modifier final concentration in the assay was as follows: 1 mM 1-benzylimidazole, 1 mM methimazole, 1 mM KCN, 10 μM deferoxamine, 2800 U/ml catalase, and 504 U/ml superoxide dismutase.

**HPLC Analyses.** HPLC analyses of supernatants were carried out on a gradient-controlled Gilson Solvent Module (Gilson, Inc., Middleton, WI) and Gilson 117 Detector using a Beckman ODS 5-μm reverse phase C18 column (4.6 mm × 25 cm; Beckman Instruments, Inc.) and a Brownlee Newguard guard column (Rainin Instruments, Woburn, MA). The flow rate was 1 ml/min with 20-μl injections made by a Gilson 234 Autoinjector. Pump A had a mobile phase of 1% ACN in water, pH adjusted to 2.5 with TFA, and pump B had a mobile phase of 75% ACN, pH adjusted to 2.5 with TFA. The detection wavelength used was 220 nm. The gradient was as follows: initial 15% B for 5 min and then increased to 40% B over a 3-min period and maintained for 5 min after which it was returned to 15% B over 2.5 min and kept for the remainder of the 20-min analysis period. This method allowed detection of the two sulfoxide diastereomers, although their exact chiral identity was not determined. The earlier eluting peak of the two diastereomers was referred to as diastereomer 1 and the later eluting peak as diastereomer 2. Retention times for the compounds of interest were TCVC, 11.5 min; and the two TCVCs diastereomers, 4.5 and 5.0 min. Quantitation of enzymatically produced TCVC was done by comparing peak areas produced to the corresponding areas of known concentrations of synthetic TCVC standards (r2 > 0.99). The limit of detection of TCVC was 25 pmol/20 μl injection. Data were corrected for nonenzymatic activity (trace amounts) by subtracting the peak areas of the 0-min and/or NADPH controls.

**In Vivo Toxicity Studies.** Male Sprague-Dawley rats (175–220 g; Sasco) were housed in individual plastic metabolic cages (Nalg Nunc International, Rochester, NY) in a controlled room on a 12-h light/dark cycle, and they were given food (Rodent laboratory chow; Harlan Teklad, Madison, WI) and water ad libitum. Rats were given a single i.p. injection of 0.6 ml of isotonic saline (control) or TCVC or TCVCs (115 or 230 μmol/kg body weight, dissolved in saline along with a few drops of 1 N NaOH; pH adjusted to approximately 8). The second series of experiments required a pretreatment (single dose i.p.) of saline or AOAA (500 μmol/kg body weight) 30 min before the administration of 230 μmol/kg TCVC. Two groups of four rats each (total of eight rats) were used in this study. All rats were sacrificed 24 h after treatment. Serum was analyzed for BUN levels, a marker for nephrotoxicity, and for SGPT and SGOT activities, as markers for hepatic damage. Urine samples were also collected and analyzed for NPT activities as another indicator of renal damage (Scherberich et al., 1994). In brief, renal or hepatic tissues were embedded in paraffin, cut, and stained with hematoxylin and eosin for qualitative assessment of nephrotoxicity or hepatoxicity without prior knowledge of the protocol to which each section belonged. For renal damage, the following criteria were used: minimal (0–25% of tubules showing necrosis), mild (25–50% of the tubules showing necrosis), moderate (50–75% of tubules showing necrosis), and severe (75–100% of tubules showing necrosis).

**Determination of Hepatic and Renal Reduced NPT and NPT Disulfides.** Reduced NPT and NPT disulfides were determined by minor modifications of a previously described method (Sausen and Elfarra, 1991; Elfarra et al., 1994). In brief, renal or hepatic tissue (1 g) was homogenized in 10 or 20 volumes of a solution of 0.15 M KCl, 30 mM EDTA, pH 4.3, respectively. An aliquot of the homogenate (2 ml) was deproteinized by adding 3 ml of a solution containing 0.3 g/ml NaCl, 0.017 g/ml metaphosphoric acid, and 0.002 g/ml EDTA. The solution was then centrifuged for 20 min at 3000 rpm and 4°C, using a Beckman TJ-6 tabletop centrifuge. The supernatants were used to determine reduced NPT and NPT disulfides as described previously (Elfarra et al., 1994).

**Statistics.** All data are expressed as means ± S.D. of measurements made on the indicated number (n) of microsomal experiments or animals as indicated in the tables or figure legends. Statistical analyses were carried out using SigmaStat (SPSS Inc., Chicago, IL). Data were analyzed using t test or Mann–Whitney rank sum test as appropriate, using p < 0.05 as the criterion for significance.

**Results**

**Characterization of TCVC Oxidation in Rat Liver and Kidney Microsomes.** Incubations of 5 mM TCVC with male rat liver or kidney microsomes at 37°C and pH 7.4 resulted in detection of two peaks by HPLC, corresponding to the two TCVCs diastereomers; the late-eluting peak (diastereomer 2) made up 60 to 100% of the total TCVC peak areas with both rat liver and kidney microsomes. TCVCs formation was dependent upon incubation time and the presence of both protein and NADPH (1.5 mM). The results presented in Fig. 2 show that TCVCs formation was linear for at least 30 min with the rate exhibited by liver microsomes being nearly 5 times higher than the rate exhibited by kidney microsomes. At a lower TCVC concentration (1 mM), TCVCs formation was detectable only with the liver microsomes (data not shown). Addition of superoxide dismutase, catalase, KCN (peroxidase inhibitor), or deferoxamine (iron chelator) did not significantly inhibit TCVCs formation, whereas inclusion of 1-benzylimi-
diazole (cytochrome P450 inhibitor) or methimazole (FMO alternate substrate) almost totally inhibited TCVC oxidation (Fig. 3), consistent with involvement of P450s and FMOs in the TCVC S-oxidase activity of rat liver microsomes. Methimazole or 1-benzylimidazole also inhibited (by nearly 80–90%) TCVC oxidation in rat kidney microsomes (data not shown).

Acute in Vivo Toxicity of TCVC and TCVCS

Physical Observations and Urine Output. Rats given TCVC or TCVCS at the 115 μmol/kg dose remained physically active throughout the 24-h study, whereas rats treated with TCVC or TCVCS at the 230 μmol/kg dose seemed lethargic within 1 h after dosing. The TCVC-treated rats exhibited improved physical condition with time until they were sacrificed 24 h post-treatment, whereas the physical condition of the TCVCS-treated rats worsened with time (they exhibited recumbency and their hind limbs seemed paralyzed) until 3 h post-treatment when their physical condition improved until they were sacrificed 24 h post-treatment. Rats given 230 μmol/kg TCVC excreted significantly less urine (9.9 ± 6.3 ml; mean ± S.D.) over the 24-h time period relative to control rats (18.9 ± 7.1 ml). However, the urine volumes of all other treatment groups were not statistically different from that of control rats.

Morphology. The liver and kidneys of rats given 115 μmol/kg TCVC or TCVCS, the livers and kidneys of rats given 230 μmol/kg TCVC, and the liver of rats given 230 μmol/kg TCVCS appeared morphologically normal. However, the kidneys of rats given 230 μmol/kg TCVCS showed visible signs of necrosis. Morphologically, the latter kidneys seemed normal in size compared with control, but they were tanncolored and when cut, there were bloody lesions in the medulla.

Histology. Histological examination of the liver sections from rats treated with both TCVC and TCVCS were normal, and they did not exhibit any lesions at the two doses given. In contrast, kidneys from rats treated with the 115 μmol/kg dose of TCVC or TCVCS showed slight-to-mild acute tubular necrosis (data not shown). Kidneys taken 2 h post-treatment from rats given TCVC (230 μmol/kg) exhibited scattered foci of mild acute tubular necrosis (Fig. 4B) compared with saline-treated animals (Fig. 4A), whereas kidneys from rats given 230 μmol/kg TCVCS showed widespread acute tubular necrosis, intratubular casts, and interstitial congestion and hemorrhage (Fig. 4C). At 24 h post-treatment, rats given 230 μmol/kg TCVCS showed mild-to-moderate acute tubular necrosis, whereas rats given the equimolar dose of TCVCS showed severe tubular necrosis (data not shown). Collectively, these histopathological results show that TCVCS nephrotoxicity occurs faster than that of TCVC, and they suggest TCVCS is also a more potent nephrotoxicant.

Clinical Chemistry. All the biochemical parameters examined from rats given 115 μmol/kg TCVC or TCVCS were similar to the corresponding parameters from control rats, except for the SGPT levels in the TCVCS-treated animals that were significantly lower than those in the saline-treated controls (Table 1). Consistent with the histopathological results described above, rats given 230 μmol/kg TCVCS exhibited a nearly 4-fold significant increase in BUN levels relative to saline-treated rats, whereas rats treated with the equimolar dose of TCVC did not exhibit a significant elevation in BUN levels (Table 1). Serum glucose levels were unaffected in rats treated with TCVC and TCVCS, whereas urine glucose levels in treated rats varied greatly among the treated animals. Three of the eight rats treated with 230 μmol/kg TCVC and four of eight rats treated with the equimolar dose of TCVCS excreted >50 mg of glucose within 24 h post-treatment, whereas control animals excreted only 0.9 ± 0.7 mg of glucose within 24 h. Large variability was also observed in GGTP levels, with five of eight rats given 230 μmol/kg TCVC and four of eight rats given the equimolar dose of TCVCS showing GGTP levels that were more than 3-fold higher than the average control value (3.4 ± 1.4 U). Interestingly, most of the TCVC- and TCVCS-treated animals that excreted >50 mg of glucose also had elevated GGTP levels, and they exhibited moderate-to-severe kidney lesions, whereas the animals that did not excrete high levels of glucose and GGTP exhibited minimal-to-mild tubular necrosis. The SGOT activities were not significantly increased with either TCVC or TCVCS treatment; however there was a significant decrease in SGPT levels in rats given TCVCS, which was similar in magnitude to that observed with the 115 μmol/kg dose. Collectively, these clinical chemistry results show that kidney injury has occurred at the high dose of both TCVC and TCVCS. However, the results suggest TCVCS is the more potent nephrotoxicant.

Hepatic and Renal Thiol Status. To help elucidate the mechanism of TCVC and TCVCS toxicity, we examined the effect of TCVC or TCVCS (230 μmol/kg) administration on liver and kidney NPT status. TCVC treatment significantly decreased the reduced NPT concentration in kidneys to approximately 52% of control values 2 h post-treatment (Table 2). The equimolar dose of TCVCS had no effect on the kidney NPT concentration. No effect was observed on the NPT disulfides in the kidney, and there was no significant alteration of NPT status in liver by either TCVC or TCVCS.

AOAA Pretreatment. The effect of the β-lyase inhibitor AOAA on TCVC toxicity at the 230 μmol/kg dose was examined. Pretreatment of rats with AOAA 30 min before TCVC treatment resulted in increased toxicity, because one of
the AOAA-TCVC-treated rats died and another had to be sacrificed before the 24-h time point, whereas animals treated with the same dose of TCVC only showed minimal discomfort.

**Discussion**

To our knowledge, the results presented here are the first to characterize TCVCs formation in rat liver and kidney microsomes. In this regard, both rat liver and kidney microsomes were able to produce TCVCs from TCVC at physiological conditions of pH 7.4 and 37°C. However, both of these activities diminished as the TCVC concentration was lowered from 5 to 1 mM, suggesting any role for TCVCs in TCVC-induced toxicity will probably increase as the hepatic and renal TCVC concentrations are increased. Because of its ability to concentrate tubular fluid and the presence of active transport carriers for cysteine S-conjugates along the nephron, any TCVC or TCVCs present in the circulation is likely to get concentrated in tubular cells to selectively predispose these cells to TCVC- and TCVCs-induced toxicities. Evidence for this hypothesis is provided by the selective nephrotoxicity of both TCVC and TCVCs after i.p. administration (Table 1; Fig. 4), and the selective depletion of renal NPT after rats were given TCVCs (Table 2). The findings that TCVC oxidation was NADPH-dependent, stereoselective, and inhibited by methimazole or 1-benzylimidazole are consistent with the involvement of FMO3 and P450s in this sulfoxidation reaction (Ripp et al., 1997, 1999; Krause et al., 2003). N-Acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine, the mercapturic acid of TCVC, is metabolized to its corresponding sulfoxide by the isozymes of the cytochrome P450 3A family (Werner et al., 1996).

The results presented here are also the first to characterize the in vivo toxicity of TCVCs in any species. The clinical chemistry, urine output, and histopathological results (Table 1; Fig. 4) revealed a dose- and time-dependent toxicity for TCVCs. The results also show the kidney was the primary target organ of TCVC toxicity and that TCVCs was a more potent nephrotoxicant than TCVC, as demonstrated by the significant increase in BUN levels in the TCVCs-treated animals. Increases in BUN levels are consistent with a drop in glomerular filtration rate secondary to either vasoconstriction or direct tubular damage or both. Urine glucose and GGTP levels were not significantly elevated in either TCVC- or TCVCs-treated animals (230 μmol/kg), but a large variability in the animal response was observed. Four of four TCVCs-treated rats and three of three TCVC-treated rats that excreted >50 mg of glucose had elevated GGTP levels. These increases in glucose excretion and GGTP levels are consistent with tubular damage as was observed histopathologically. Collectively, these results show that although both TCVC and TCVCs can induce nephrotoxicity, TCVCs is a more potent nephrotoxin than TCVC. When rats were pretreated with AOAA before TCVC, they exhibited severe toxicity and two rats of four died during the course of the 24-h experiment, whereas all rats given the equimolar dose of TCVC did not exhibit severe toxicity signs or death during the course of the experiment. This was unexpected, because this AOAA dosing protocol had been found previously protective with other nephrotoxic cysteine S-conjugates (Lash et al., 1994; Elfarra, 1997; Krause et al., 2002). Because of the
potent nephrotoxicity of TCVCS compared with TCVC, the data suggest that inhibiting the β-lyase pathway results in increased toxicity, possibly due to increased formation of TCVCS in rats given AOAA and TCVC. This finding also suggests that even a small flux through the S-oxidase pathway may account for the increased toxic effects seen with TCVCS. Interestingly, sulfoxidation was previously shown to increase the toxicity of N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine, the mercapturic acid of TCVCS, in rat renal proximal tubular cells in vitro (Werner et al., 1996).

Although TCVCS administration was not associated with any histopathological effects on the liver, a significant decrease in SGPT levels was observed with both the high and low TCVCS doses (Table 1). Because TCVC readily reacts in vitro with glutathione (t1/2 = 20 min), although TCVC is not reactive (Ripp et al., 1997), TCVCS may have reacted with a reactive nucleophilic group on the enzyme, causing inhibition of the SGPT activity. A similar decrease in SGPT activity was reported after rats were treated with methadithion (Altuntas et al., 2003). The temporary loss of motor activity in the lower part of the spinal cord, ion channels, or cholinergic receptors in muscles secondary to diffusion of some TCVCS after its i.p. administration.

The in vivo results observed with TCVCS are for the most part similar to the results that we have reported previously with S-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS) (Sausen and Elfarra, 1991; Lash et al., 1994). Although TCVCS was a potent nephrotoxin similar to DCVCS (Lash et al., 1994), TCVCS depleted kidney NPT, and it had no effect on liver NPT status, whereas DCVCS depleted NPT in both the liver and kidney. These differences between TCVCS and DCVCS could be explained by the lower reactivity of TCVCS toward biological nucleophiles in comparison with DCVCS (Ripp et al., 1997), which may have allowed TCVCS to be more selective for the kidneys.

Although we did not characterize TCVCS metabolism by human FMOs, we have previously shown that human FMOs exhibit kinetic values with L-methionine, S-benzyl-L-cysteine, and S-allyl-L-cysteine similar to those exhibited by rat and rabbit FMOs (Duescher et al., 1994; Ripp et al., 1997, 1999). This suggests human FMO3 may also convert TCVCS to TCVCS. FMO3 is the major FMO isoform expressed in human enzymes have recently been shown to convert DCVC to DCVCS (Krause et al., 2003), and as little as 10 μM DCVCS has been shown to induce cytotoxicity in primary cultures of human renal proximal tubular cells.

TABLE 1
Effects of TCVCS and TCVCs sulfoxide on renal and hepatic function in male Sprague-Dawley rats
Male Sprague-Dawley rats (175–225 g) were given a single i.p. injection of isotonic saline, TCVC, or TCVCS. Animals were sacrificed 2 h later, and NPT concentrations were analyzed as described under Materials and Methods. Values are means ± S.D. (n = 4–8).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>BUN</th>
<th>Serum Glucose</th>
<th>SGOTa</th>
<th>SGPTa</th>
<th>Total Urinary Glutamateb</th>
<th>Total Urinary Glucoseb</th>
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<td>135.7±46.4</td>
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<td>TCVC 115 μmol/kg</td>
<td>8</td>
<td>23.0±4.0</td>
<td>126.8±10.5</td>
<td>111.0±15.9</td>
<td>39.0±8.4</td>
<td>5.5±1.6</td>
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<tr>
<td>TCVC 230 μmol/kg</td>
<td>8</td>
<td>28.7±10.8</td>
<td>148.2±21.2</td>
<td>193.6±74.7</td>
<td>55.1±20.0</td>
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<td>56.0±73.7</td>
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<tr>
<td>TCVCS</td>
<td></td>
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<tr>
<td>115 μmol/kg</td>
<td>8</td>
<td>25.2±5.0</td>
<td>132.7±7.8</td>
<td>122.5±23.5</td>
<td>21.8±7.1**</td>
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<tr>
<td>230 μmol/kg</td>
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<td>96.1±52.7***</td>
<td>153.7±23.7</td>
<td>204.8±105.6</td>
<td>28.0±10.3*</td>
<td>10.3±12.0</td>
<td>46.2±54.1</td>
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* Statistically different from saline-treated rats; p < 0.05 as determined by t test or Mann-Whitney rank sum test as appropriate.
** Statistically different from saline-treated rats; p < 0.01 as determined by t test or Mann-Whitney rank sum test as appropriate.
*** Significantly different from saline-treated rats; p < 0.001 as determined by t test or Mann-Whitney rank sum test as appropriate.
† One SF unit (SF U) will form 4.82 × 10⁻⁴ μmol glutamate/min at pH 7.5 and 25°C.
‡ One unit (U) is equal to 1 μmol product/min at 25°C.

TABLE 2
Effects of TCVCS and TCVCs (230 μmol/kg) on NPT status in rats 2 h post-treatment
Male Sprague Dawley rats (160–175 g) were given a single i.p. injection of either saline, TCVC, or TCVCS. Animals were sacrificed 2 h later, and NPT concentrations were determined as described under Materials and Methods. Values are means ± S.D. (n = 4).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver Reduced NPT</th>
<th>NPT Disulfides μmol/g tissue</th>
<th>Kidney Reduced NPT</th>
<th>NPT Disulfides μmol/g tissue</th>
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</thead>
<tbody>
<tr>
<td>Saline</td>
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<td>TCVC 115 μmol/kg</td>
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<td>TCVCS 230 μmol/kg</td>
<td>6.5±1.3</td>
<td>0.3±0.2</td>
<td>1.8±0.6*</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

** Statistically significant from saline-treated rats (p < 0.01).
Nephrototoxicity of TCVC and Its Corresponding Sulfoxide

(Chen et al., 2003), TCVC could also be oxidized in human tissues to yield TCVCS, which could then contribute to TCVC toxicity in human cells.

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