

# 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 monooxygenases (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 the rate measured with rat kidney microsomes, whereas 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  $\mu\text{mol/kg}$  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 work-

ers in the dry cleaning industry (International Agency for Research on Cancer, 1995). Additional epidemiological studies have shown increases in kidney cancers (Katz and Jowett, 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,  $\beta$ -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  $\text{mg/m}^3$ ) 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

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**ABBREVIATIONS:** TETRA, tetrachloroethylene or tetrachloroethene; P450, cytochrome P450; TCVG, S-(1,2,2-trichlorovinyl)glutathione; TCVC, S-(1,2,2-trichlorovinyl)-L-cysteine; GGTP,  $\gamma$ -glutamyl transpeptidase;  $\beta$ -lyase, cysteine conjugate  $\beta$ -lyase; FMO, flavin-containing monooxygenase; TCVCS, S-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide; AOAA, aminooxyacetic acid; NPT, nonprotein thiol; SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamate-oxalacetate transaminase; BUN, blood urea nitrogen; TFA, trifluoroacetic acid; HPLC, high-pressure liquid chromatography; ACN, acetonitrile.

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)glutathione (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  $\gamma$ -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  $\beta$ -lyases ( $\beta$ -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, TCVCS has been shown to be a substrate for FMO3, but not for FMO1, FMO2, or FMO5. Because TCVCS reacts readily with glutathione ( $t_{1/2} = 20$  min) in vitro, but TCVC is unreactive (Ripp et al., 1997), TCVCS formation has been implicated in TCVC-induced nephrotoxicity.

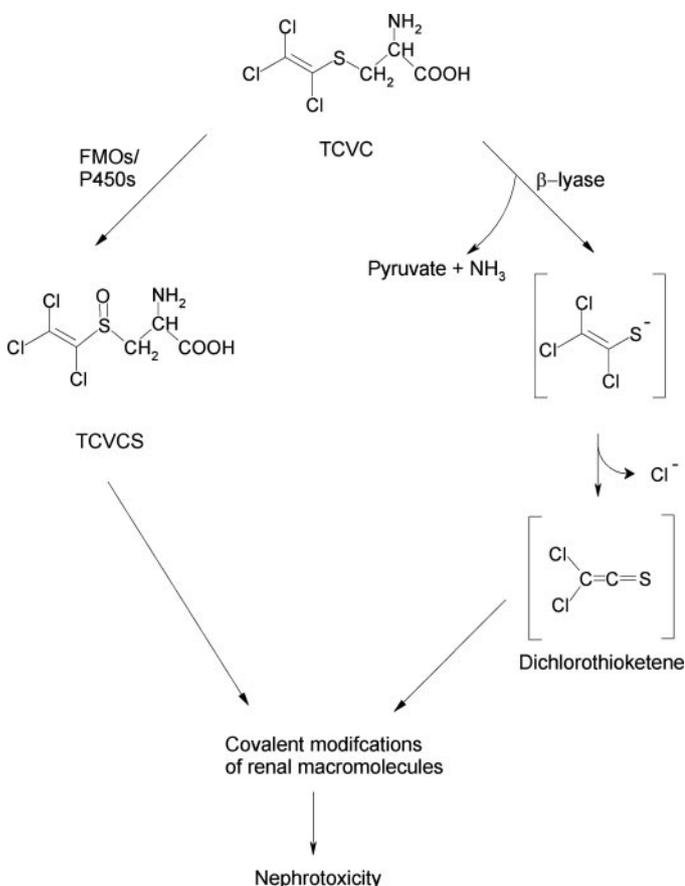
In the current study, we characterized the ability of rat liver and kidney microsomes to metabolize TCVC to TCVCS in vitro. We also investigated TCVCS nephrotoxicity in rats in vivo and determined the effect of aminooxyacetic acid (AOAA), a potent  $\beta$ -lyase inhibitor, on TCVC toxicity. Furthermore, 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  $\beta$ -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). Kits to determine serum glutamate-pyruvate transaminase (SGPT), serum glutamate-oxalacetate transaminase (SGOT), and urinary GGTP 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 NaBH<sub>4</sub> 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 KH<sub>2</sub>PO<sub>4</sub>, 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  $\mu$ l) 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 kidney microsomal experiments were carried out in the presence of 0.1 mM AOAA to inhibit any  $\beta$ -lyase-mediated reaction (Lash et al., 1994; Werner et al., 1996). To terminate the reaction, ice-cold 0.75% perchloric acid (0.15 ml) was added. The samples were centrifuged 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- $\mu$ m 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  $\mu$ M 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- $\mu$ m reverse phase C18 column (4.6 mm  $\times$  25 cm; Beckman Instruments, Inc.) and a Brownlee Newguard guard column (Rainin Instruments, Woburn, MA). The flow rate was 1 ml/min with 20- $\mu$ l 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 is 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 TCVCS was done by comparing peak areas produced to the corresponding areas of known concentrations of synthetic TCVCS standards ( $r > 0.99$ ). The limit of detection of TCVCS was 25 pmol/20  $\mu$ l 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 (Nalge 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 (0.6 ml) of isotonic saline (control) or TCVC or TCVCS (115 or 230  $\mu$ mol/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  $\mu$ mol/kg body weight) 30 min before the administration of 230  $\mu$ mol/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 liver injury. Urine samples were also collected and analyzed for GGTP activity as another indicator of renal damage (Scherberich et al., 1974). Furthermore, serum and urine samples were analyzed for glucose levels.

In a third series of experiments designed to provide insight into the mechanism of TCVC- and TCVCS-induced toxicity, renal and hepatic reduced NPT and NPT disulfide concentrations were measured. In total, 12 animals divided into three treatment groups of four rats each were used. In brief, a single injection of saline, TCVC, or TCVCS (230  $\mu$ mol/kg) was given i.p., and the animals were sacrificed 2 h after treatment. Portions of the kidneys and livers were removed for NPT and NPT disulfide determination according to the method described below.

Evaluation of renal and hepatic damage by histopathological analysis was carried out at the histopathology facility of the Veterinary Medical Teaching Hospital, University of Wisconsin (Madison, WI). Tissues were fixed in 10% buffered formalin. After fixation, the tissues were embedded in paraffin, cut, and stained with hematoxylin and eosin for qualitative assessment of nephrotoxicity or hepatotoxicity 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).

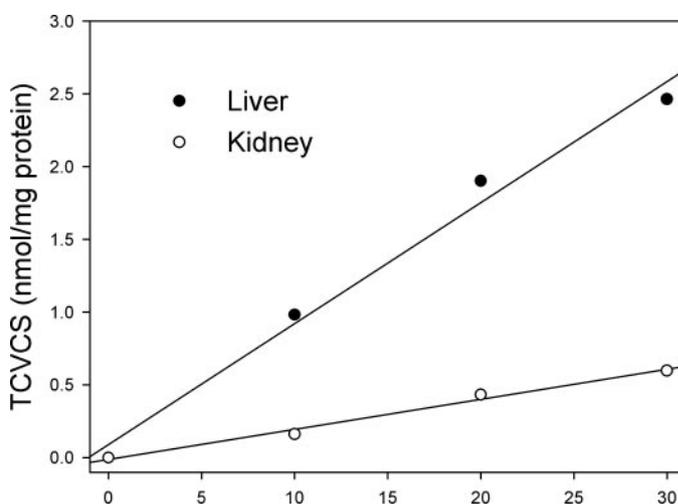
**Determination of Hepatic and Renal Reduced NPT and NPT Disulfides.** Reduced NPT and NPT disulfides were determined by minor modifications of a previously described method (Sausen and Elfarra, 1991; Elfarra et al., 1994). In brief, renal or hepatic tissue (1 g) was homogenized in 10 or 20 volumes of 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  $\pm$  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 TCVCS 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-benzylimidazole



**Fig. 2.** Representative results from time-dependent oxidation of TCVC to TCVCS by male rat liver and kidney microsomes. Experiments were carried out as described under *Materials and Methods*, using 5 mM TCVC and 1.5 mM NADPH.

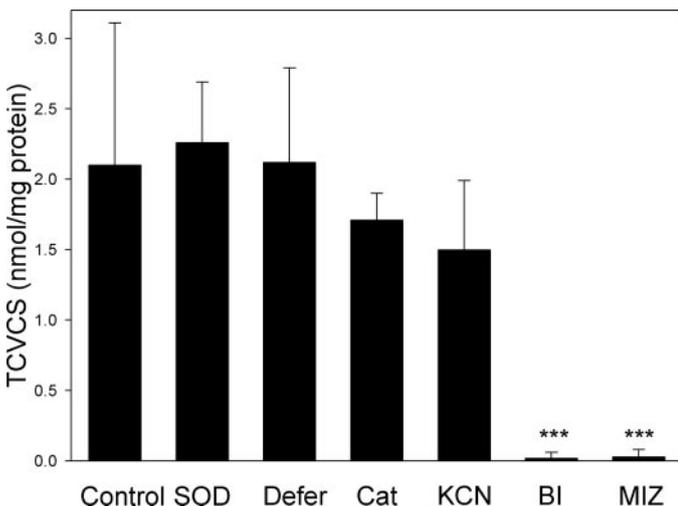
dazole (cytochrome P450 inhibitor) or methimazole (FMO alternate substrate) almost totally inhibited TCVC oxidation (Fig. 3), consistent with involvement of P450s and FMOs in the TCVC *S*-oxidase activity of rat liver microsomes. Methimazole or 1-benzylimidazole also inhibited (by nearly 80–90%) TCVC 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  $\mu\text{mol/kg}$  dose remained physically active throughout the 24-h study, whereas rats treated with TCVC or TCVCS at the 230  $\mu\text{mol/kg}$  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  $\mu\text{mol/kg}$  TCVC excreted significantly less urine ( $9.9 \pm 6.3$  ml; mean  $\pm$  S.D.) over the 24-h time period relative to control rats ( $18.9 \pm 7.1$  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  $\mu\text{mol/kg}$  TCVC or TCVCS, the livers and kidneys of rats given 230  $\mu\text{mol/kg}$  TCVC, and the liver of rats given 230  $\mu\text{mol/kg}$  TCVCS seemed morphologically normal. However, the kidneys of rats given 230  $\mu\text{mol/kg}$  TCVCS showed visible signs of necrosis. Morphologically, the latter kidneys seemed normal in size compared with control, but they were tan-colored 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  $\mu\text{mol/kg}$  dose of TCVC or TCVCS showed slight-to-mild acute tubular



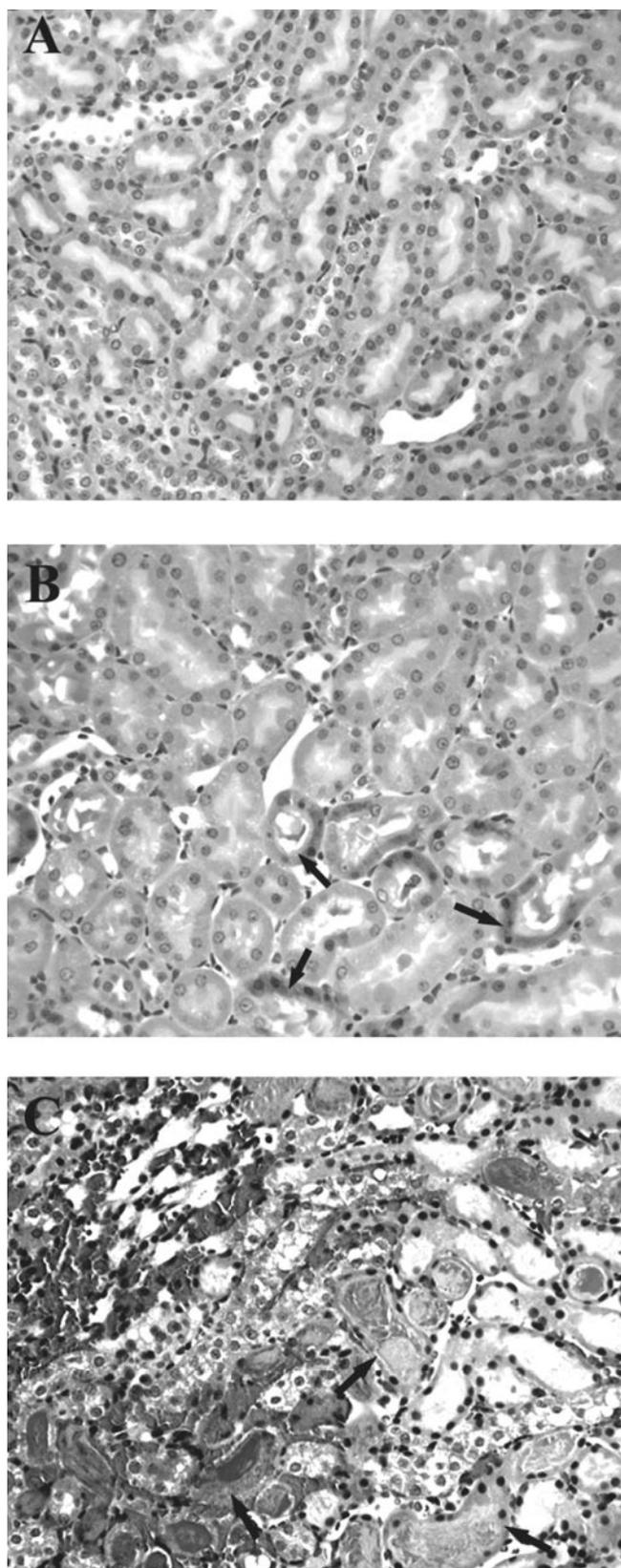
**Fig. 3.** Effect of inhibitors, alternative substrates, and oxygen scavengers on TCVCS formation. All activities were determined as described under *Materials and Methods* using 5 mM TCVC, 1.5 mM NADPH, and male rat liver microsomes for 30 min. All activities are corrected for non-NADPH-dependent TCVCS formation. Values (means  $\pm$  S.D.;  $n = 3$ ) marked with asterisks are significantly different from control ( $p < 0.001$ ).

necrosis (data not shown). Kidneys taken 2 h post-treatment from rats given TCVC (230  $\mu\text{mol/kg}$ ) exhibited scattered foci of mild acute tubular necrosis (Fig. 4B) compared with saline-treated animals (Fig. 4A), whereas kidneys from rats given 230  $\mu\text{mol/kg}$  TCVCS showed widespread acute tubular necrosis, intratubular casts, and interstitial congestion and hemorrhage (Fig. 4C). At 24 h post-treatment, rats given 230  $\mu\text{mol/kg}$  TCVC 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  $\mu\text{mol/kg}$  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  $\mu\text{mol/kg}$  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  $\mu\text{mol/kg}$  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  $0.9 \pm 0.7$  mg of glucose within 24 h. Large variability was also observed in GGTP levels, with five of eight rats given 230  $\mu\text{mol/kg}$  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 ( $3.4 \pm 1.4$  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  $\mu\text{mol/kg}$  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  $\mu\text{mol/kg}$ ) administration on liver and kidney NPT status. TCVCS 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 TCVC 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  $\beta$ -lyase inhibitor AOAA on TCVC toxicity at the 230  $\mu\text{mol/kg}$  dose was examined. Pretreatment of rats with AOAA 30 min before TCVC treatment resulted in increased toxicity, because one of



**Fig. 4.** Representative histopathological kidney sections (all 200 $\times$ ) from rats treated with saline (A), 230  $\mu\text{mol/kg}$  TCVC (B), or 230  $\mu\text{mol/kg}$  TCVCS (C) and killed 2 h after dosing. Note the scattered foci of mild acute tubular necrosis (arrows) in B and the widespread acute tubular necrosis (regionally severe in deep cortex and outer stripe of the outer medulla), intratubular casts (arrows), and interstitial congestion and hemorrhage in C.

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 TCVCS 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  $\mu\text{mol/kg}$ ), 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; Elfarrar, 1997; Krause et al., 2002). Because of the



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

#### Acknowledgments

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#### References

- Altuntas I, Delibas N, Demirci M, Kilinc I, and Tamer N (2002) The effects of methidathion on lipid peroxidation and some liver enzymes: role of vitamins E and C. *Arch Toxicol* **76**:470–473.
- Anna CH, Maronpot RR, Pereira MA, Foley JF, Markley DE, and Anderson MW (1994) Ras proto-oncogene activation in dichloroacetic acid-, trichloroethylene- and tetrachloroethylene-induced liver tumors in B6C3F1 mice. *Carcinogenesis* **15**: 2255–2261.
- Antilla A, Pukkala E, Sallmen M, Hernberg S, and Hemminki K (1995) Cancer incidence among Finnish workers exposed to halogenated hydrocarbons. *J Occup Med* **37**:797–806.
- Birner G, Rutkowska A, and Dekant W (1996) *N*-Acetyl-*S*-(1,2,2-trichlorovinyl)-*L*-cysteine and 2,2,2-trichloroethanol. Two novel metabolites of tetrachloroethene in humans after occupational exposure. *Drug Metab Dispos* **24**:41–48.
- Dekant W, Metzler M, and Henschler D (1986) Identification of *S*-(1,2,2-trichlorovinyl)-*N*-acetylcysteine as a urinary metabolite of tetrachloroethylene: bioactivation through glutathione conjugation as a possible explanation of its nephrocarcinogenicity. *J Biochem Toxicol* **1**:57–72.
- Dekant W, Berthold K, Vamvakas S, Henschler D, and Anders MW (1988) Thioacylating intermediates as metabolites of *S*-(1,2-dichlorovinyl)-*L*-cysteine and *S*-(1,2,2-trichlorovinyl)-*L*-cysteine formed by cysteine conjugate  $\beta$ -lyase. *Chem Res Toxicol* **1**:175–178.
- Dekant W, Martens G, Vamvakas S, Metzler M, and Henschler D (1987) Bioactivation of tetrachloroethylene: role of glutathione *S*-transferase-catalyzed conjugation versus cytochrome P-450-dependent phospholipid alkylation. *Drug Metab Dispos* **15**:702–709.
- Duescher RJ, Lawton MP, Philpot RM, and Elfarra AA (1994) Flavin-containing monooxygenase (FMO)-dependent metabolism of methionine and evidence for FMO3 being the major FMO involved in methionine sulfoxidation in rabbit liver and kidney microsomes. *J Biol Chem* **269**:17525–17530.
- Duh RW and Asal NR (1984) Mortality among laundry and dry cleaning workers in Oklahoma. *Am J Public Health* **74**:1278–1280.
- Elfarra AA (1997) Halogenated hydrocarbons, in *Comprehensive Toxicology* (Goldstein RS ed) vol. 7, pp 601–616. Elsevier Science, New York.
- Elfarra AA, Duescher RJ, Sausen PJ, O'Hara TM, and Cooley AJ (1994) Methimazole protection of rats against gentamicin-induced nephrotoxicity. *Can J Physiol Pharmacol* **72**:1238–1244.
- Franchini I, Cavotorta A, Falzoi M, Lucertini S, and Mutti A (1983) Early indicators of renal damage in workers exposed to organic solvents. *Int Arch Occup Environ Health* **52**:1–9.
- Green T, Odum J, Nash JA, and Foster JR (1990) Perchloroethylene-induced rat kidney tumors: an investigation of the mechanisms involved and their relevance to humans. *Toxicol Appl Pharmacol* **103**:77–89.
- International Agency for Research on Cancer (1995) Dry cleaning, some chlorinated solvents and other industrial chemicals, in *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, vol. 63, pp 159–221, International Agency for Research on Cancer, Lyon, France.
- Katz RM and Jowett D (1981) Female laundry and dry cleaning workers in Wisconsin: mortality analysis. *Am J Public Health* **71**:305–307.
- Krause RJ, Glocke SC, and Elfarra AA (2002) Sulfoxides as urinary metabolites of *S*-allyl-*L*-cysteine in rats: evidence for the involvement of flavin-containing monooxygenases. *Drug Metab Dispos* **30**:1137–1142.
- Krause RJ, Lash LH, and Elfarra AA (2003) Human kidney flavin-containing monooxygenases (FMOs) and their potential roles in cysteine *S*-conjugate metabolism and nephrotoxicity. *J Pharmacol Exp Ther* **304**:185–191.
- Lash LH, Nelson RM, Van Dyke RA, and Anders MW (1990) Purification and characterization of human kidney cystolic cysteine conjugate  $\beta$ -lyase activity. *Drug Metab Dispos* **18**:50–54.
- Lash LH, Sausen PJ, Duescher RJ, Cooley AJ, and Elfarra AA (1994) Roles of cysteine conjugate  $\beta$ -lyase and *S*-oxidase in nephrotoxicity: studies with *S*-(1,2-dichlorovinyl)-*L*-cysteine and *S*-(1,2-dichlorovinyl)-*L*-cysteine sulfoxide. *J Pharmacol Exp Ther* **269**:374–383.
- Lash LH and Parker JC (2001) Hepatic and renal toxicities associated with perchloroethylene. *Pharmacol Rev* **53**:177–208.
- Lash LH, Qian W, Putt DA, Desai K, Elfarra AA, Sicuri AR, and Parker JC (1998) Glutathione conjugation of perchloroethylene in rats and mice in vitro: sex-, species-, and tissue-dependent differences. *Toxicol Appl Pharmacol* **150**:49–57.
- Lash LH, Qian W, Putt DA, Hueni SE, Elfarra AA, Sicuri AR, and Parker JC (2002) Renal toxicity of perchloroethylene and *S*-(1,2,2-trichlorovinyl)glutathione in rats and mice: sex- and species-dependent differences. *Toxicol Appl Pharmacol* **179**: 163–171.
- Lash LH, Putt DA, Hueni SE, Krause RJ, and Elfarra AA (2003) Roles of necrosis, apoptosis, and mitochondrial dysfunction in *S*-(1,2-dichlorovinyl)-*L*-cysteine sulfoxide-induced cytotoxicity in primary cultures of human renal proximal tubular cells. *J Pharmacol Exp Ther* **305**:1163–1172.
- Lowry OH, Rosenbrough NJ, Farr AL, and Randall RJ (1951) Protein measurements with the folin phenol reagent. *J Biol Chem* **193**:265–275.
- Mutti A, Alinovi R, Bergamaschi E, Biagini C, Cavazzini S, Franchini I, Lauwerys RR, Bernard AM, Roels H, Gelpi E, et al. (1992) Nephropathies and exposure to perchloroethylene in dry-cleaners. *Lancet* **340**:189–193.
- National Toxicology Program (1986) Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies). Department of Health and Human Services-National Institutes of Health Report 86-2567. National Toxicology Program, National Institutes of Health, Bethesda, MD.
- Pähler A, Parker J, and Dekant W (1999) Dose-dependent protein adduct formation in kidney, liver, and blood of rats and in human blood after perchloroethene inhalation. *Toxicol Sci* **48**:5–13.
- Ripp SL, Itagaki K, Philpot RM, and Elfarra AA (1999) Species and sex differences in expression of flavin-containing monooxygenase form 3 in liver and kidney microsomes. *Drug Metab Dispos* **27**:46–52.
- Ripp SL, Overby LH, Philpot RM, and Elfarra AA (1997) Oxidation of cysteine *S*-conjugates by rabbit liver microsomes and cDNA-expressed flavin containing monooxygenases: studies with *S*-(1,2 dichlorovinyl)-*L*-cysteine, *S*-(1,2,2 trichlorovinyl)-*L*-cysteine, *S*-allyl-*L*-cysteine and *S*-benzyl-*L*-cysteine. *Mol Pharmacol* **51**:507–515.
- Sausen PJ and Elfarra AA (1990) Cysteine conjugate *S*-oxidase. *J Biol Chem* **265**: 6139–6145.
- Sausen PJ and Elfarra AA (1991) Reactivity of cysteine *S*-conjugate sulfoxides: formation of *S*-[1-chloro-2-(*S*-glutathionyl)vinyl]-*L*-cysteine sulfoxide by reaction of *S*-(1,2-dichlorovinyl)-*L*-cysteine sulfoxide with glutathione. *Chem Res Toxicol* **4**:655–660.
- Scherberich JE, Falkenberg FW, Mondorf AW, Müller H, and Pfeleiderer G (1974) Biochemical and immunological studies on isolated brush border membranes of human kidney cortex and their membrane surface proteins. *Clin Chim Acta* **55**:179–197.
- Vamvakas S, Herkenhoff M, Dekant W, and Henschler D (1989a) Mutagenicity of tetrachloroethene in the Ames test: metabolic activation by conjugation with glutathione. *J Biochem Toxicol* **4**:21–27.
- Vamvakas S, Kochling A, Berthold K, and Dekant W (1989b) Cytotoxicity of cysteine *S*-conjugates: structure-activity relationships. *Chem Biol Interact* **71**:79–90.
- Verplanke AJW, Leumens MHL, and Herber RFM (1999) Occupational exposure to tetrachloroethene and its effects on the kidneys. *J Occup Environ Med* **41**:11–16.
- Völkel W, Friedewald M, Lederer E, Pähler A, Parker J, and Dekant W (1998) Biotransformation of perchloroethene: dose-dependent excretion of trichloroacetic acid, dichloroacetic acid, and *N*-acetyl-*S*-(trichlorovinyl)-*L*-cysteine in rats and humans after inhalation. *Toxicol Appl Pharmacol* **153**:20–27.
- Werner M, Birner G, and Dekant W (1996) Sulfoxidation of mercapturic acids derived from tri- and tetrachloroethene by cytochromes P450 3A: a bioactivation reaction in addition to deacetylation and cysteine conjugate  $\beta$ -lyase mediated cleavage. *Chem Res Toxicol* **9**:41–49.

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