Published in final edited form as:
*Toxicology*. 2013 April 5; 0: 157–161. doi:10.1016/j.tox.2013.02.003.

# Mutagenicity of the Cysteine *S*-Conjugate Sulfoxides of Trichloroethylene and Tetrachloroethylene in the Ames Test

**Roy M. Irving**[†] and **Adnan A. Elfarra**[†,‡,*]

[†]Molecular and Environmental Toxicology Center, University of Wisconsin-Madison, Madison, WI 53706

[‡]Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI 53706

## Abstract

The nephrotoxicity and nephrocarcinogenicity of trichloroethylene (TCE) and tetrachloroethylene (PCE) are believed to be mediated primarily through the cysteine *S*-conjugate β-lyase-dependent bioactivation of the corresponding cysteine *S*-conjugate metabolites *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) and *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), respectively. DCVC and TCVC have previously been demonstrated to be mutagenic by the Ames Salmonella mutagenicity assay, and reduction in mutagenicity was observed upon treatment with the β-lyase inhibitor aminooxyacetic acid (AOAA). Because DCVC and TCVC can also be bioactivated through sulfoxidation to yield the potent nephrotoxicants *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS) and *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide (TCVCS), respectively, the mutagenic potential of these two sulfoxides was investigated using the Ames *S. typhimurium* TA100 mutagenicity assay. The results show both DCVCS and TCVCS were mutagenic, and TCVCS exhibited 3-fold higher mutagenicity than DCVCS. However, DCVCS and TCVCS mutagenic activity was approximately 700-fold and 30-fold lower than DCVC and TCVC, respectively. DCVC and DCVCS appeared to induce toxicity in TA100, as evidenced by increased microcolony formation and decreased mutant frequency above threshold concentrations. TCVC and TCVCS were not toxic in TA100. The toxic effects of DCVC limited the sensitivity of TA100 to DCVC mutagenic effects and rendered it difficult to investigate the effects of AOAA on DCVC mutagenic activity. Collectively, these results suggest that DCVCS and TCVCS exerted a definite but weak mutagenicity in the TA100 strain. Therefore, despite their potent nephrotoxicity, DCVCS and TCVCS are not likely to play a major role in DCVC or TCVC mutagenicity in this strain.

### Keywords

chlorinated hydrocarbons; mutagenicity; *S*-(1,2-dichlorovinyl)-L-cysteine; *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide; *S*-(1,2,2-trichlorovinyl)-L-cysteine; *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide



[*]To whom correspondence should be addressed: School of Veterinary Medicine, 2015 Linden Drive, Madison, WI 53706, Tel: 608-262-6518, Fax: 608-262-3926, elfarra@svm.vetmed.wisc.edu.

**Conflict of Interest Statement**

The authors declare that there are no conflicts of interest.

## 1. Introduction

Trichloroethylene (TCE) and tetrachloroethylene (PCE; also known as perchloroethylene and abbreviated Perc or Tetra), halogenated hydrocarbons used as organic solvents, are common groundwater contaminants. Long term exposure to TCE (Brüning et al., 1996, 1998, 1999; Vermeulen et al., 2012) and PCE are known to cause nephrotoxicity (Lash and Parker, 2001) and both are classified as "reasonably anticipated to be a human carcinogen" by the National Toxicology Program (National Toxicology Program, 2011). There is an association between TCE exposure and the development of renal cancer in humans. Exposure to PCE has been associated with the development of esophageal cancer, cervical cancer, and non-Hodgkin's lymphoma. TCE and PCE are known to be renal carcinogens in rats.

The nephrotoxicity and nephrocarcinogenicity of TCE and PCE are attributed to metabolites formed by the metabolic pathway that is initiated by GSH *S*-transferases (Dekant et al., 1986, 1987, 1994; Lash et al., 1998, 2000; Lash and Parker, 2001). In this pathway, TCE and PCE are initially conjugated to GSH in the liver. The GSH conjugates are then processed further into cysteine *S*-conjugates (Figure 1), *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC; derived from TCE) and *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC; derived from PCE) in the kidney, bile duct epithelium, intestinal lumen or bile canalicular membrane of hepatocytes. DCVC and TCVC can enter the circulation and translocate to the kidneys. The mercapturic acids of DCVC and TCVC, *N*-acetyl DCVC and *N*-acetyl TCVC, are formed by *N*-acetylation in the kidney or liver and have been detected in the urine of humans exposed to TCE (Bernauer et al., 1996) or PCE (Birner et al., 1996; Völkel et al., 1998), respectively.

Two bioactivation mechanisms have been elucidated for DCVC and TCVC. Both compounds can undergo β-elimination by cysteine *S*-conjugate β-lyases (β-lyase) to form reactive electrophilic sulfur species (Elfarra et al., 1986; Lash and Anders, 1986; Dekant et al., 1988, 1994; Vamvakas et al., 1989a, b; Pähler et al., 1999a). Alternatively, DCVC and TCVC can be oxidized to form the Michael acceptor reactive sulfoxides, *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS; derived from TCE) and *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide (TCVCS; derived from PCE) (Figure 1). DCVC can be oxidized by flavin-containing monooxygenases (FMOs) (Ripp et al., 1997; Krause et al., 2003) and TCVC can be oxidized by FMOs or cytochrome P450s (Ripp et al., 1997). DCVCS (Lash et al., 1994) and TCVCS (Elfarra and Krause, 2007) have both been demonstrated to be more nephrotoxic than the precursor cysteine *S*-conjugates, likely due to their ability to act as direct-acting toxicants through covalent reactions with sulfhydryl-containing molecules. Recently, DCVCS-hemoglobin adducts and cross-links have been detected in rats given DCVC, providing evidence for *in vivo* metabolism of DCVC to DCVCS (Barshteyn and Elfarra, 2009).

In addition to their nephrotoxicity, DCVC and TCVC have been demonstrated to be mutagenic by the Ames Salmonella mutagenicity assay (Vamvakas et al., 1988; Dekant et al., 1986). DCVC and TCVC were suggested to induce base-pair substitution mutations based upon the potent effect observed in the base-pair substitution strain TA100 and the low effects in the frame-shift strain TA98 (Dekant et al., 1986). The addition of aminooxyacetic acid (AOAA), a potent inhibitor of β-lyase, reduced the mutagenicity of DCVC and TCVC, implicating the β-lyase derived metabolites in the mutagenicity of these compounds. However, the ability of DCVCS and TCVCS to also contribute to the mutagenicity of DCVC and TCVC remains unknown. Therefore, the present study sought to investigate the mutagenicity of DCVCS and TCVCS, and compare the mutagenic potency of these compounds to the mutagenic potency of DCVC and TCVC.

## 2. Materials and Methods

### 2.1 Materials

DCVC, DCVCS, TCVC, and TCVCS were synthesized as previously described (Sausen and Elfarra, 1991; Ripp et al., 1997). Purity of the synthesized chemicals was demonstrated to be > 95% by HPLC. *Salmonella typhimurium* strain TA100 was obtained from Bioreliance (Rockville, MD).

### 2.2 Assessing DCVCS and TCVCS Mutagenicity by the Ames Salmonella Mutagenicity Assay

The mutagenicity of DCVCS and TCVCS was characterized and compared to their precursor cysteine *S*-conjugates, DCVC and TCVC, utilizing the *S. typhimurium* tester strain TA100 in the Ames salmonella mutagenicity assay. This strain was chosen for the present study because it has been previously used to characterize the mutagenicity of DCVC, TCVC, and the related cysteine *S*-conjugates of hexachloro-1,3-butadiene (Dekant et al., 1986; Vamvakas et al., 1988). In addition, crotonaldehyde and acrolein, which like DCVCS and TCVCS are Michael acceptors, were mutagenic in TA100 (Jha et al., 2007; Parent et al., 1996), suggesting that Michael acceptors may induce mutagenicity through base-pair substitutions. Furthermore, DCVC and TCVC were demonstrated to be mutagenic in this strain without needing the addition of rat kidney or liver microsomes. The assay was conducted following previously described protocols (Mortelmans and Zeiger, 2000). Briefly, a culture of TA100 was grown overnight in nutrient broth containing 24μg/mL ampicilin to 1–2 × $10^9$ CFU/mL ($OD_{540}$ 0.1 – 0.2). An aliquot of the overnight culture (0.05 – 0.1 mL; 1–2 × $10^8$ cells/plate final concentration) was added to a test tube containing 0.1 mM sodium phosphate buffer pH 7.4 (0.5 mL) and test chemical dissolved in buffer (50 μL). The final concentration ranges for the test chemicals were: DCVC, 0 – 25 nmol/plate; DCVCS, 0 – 4000 nmol/plate; TCVC, 0 – 150 nmol/plate; and TCVCS, 0 – 500 nmol/plate. Sodium azide (1.33 μg/plate) was used as a positive control. Samples were incubated for 20 min at 37°C. Molten top agar (2 mL; 0.6 % Agar, 0.5% NaCl, 0.05 mM biotin, 0.05 mM histidine maintained at 43 to 48°C) was added to each tube. Samples were mixed and transferred to plates (1.5% agar, 2% glucose in Vogel Bonner medium E). After top agar hardened, plates were inverted and incubated at 37°C for 48 h, after which colonies were counted. The spontaneous mutant frequency ranged from 90 – 150 revertants per plate, which is within established acceptable control value ranges for TA100 (Mortelmans and Zeiger, 2000). All test chemical concentrations were assayed in triplicate and differences between analogous plates did not exceed 30 %. Mutagenic activity was expressed as revertants per nmol of chemical and calculated using the linear portion of the plot of revertants versus nmol of test chemical per plate. Points where toxicity was observed were not included, and as a result, the linear range was presumed to suggest no significant toxicity.

In addition, the effects of AOAA, a potent inhibitor of β-lyase, on DCVCS mutagenicity was investigated. In these experiments, AOAA (1 mM final concentration) was added to diluted cultures of TA100 (1 – 2 × $10^8$ cells/plate) along with DCVC (0 – 25 mM final concentration), and the mutagenicity assay was carried out as described above. Statistical analysis was performed using the Wilcoxon rank sum test (Mstat, http://www.mcardle.wisc.edu/mstat/) to compare mutagenic activity data from TA100 exposed to DCVC alone or DCVC and AOAA. Results were considered significant if $p<0.05$.

## 3. Results

### 3.1 Assessing DCVCS and TCVCS Mutagenicity by the Ames Salmonella Mutagenicity Assay

DCVCS (Figure 2B) and TCVCS (Figure 3B) were definite but weak mutagens in the TA100 tester strain in the Ames assay. The two sulfoxides exhibited different mutagenic activity; TCVCS mutagenic activity (0.32 revertants/nmol) was 3-fold higher than DCVCS (0.1 revertants/nmol). DCVC (76 revertants/nmol) and TCVC (11 revertants/nmol) had higher mutagenic activity compared to their corresponding sulfoxides (Figure 2A; 3A). In addition, DCVC and DCVCS appeared to induce toxicity as indicated by the increased detection of microcolonies and decreasing numbers of revertants above certain threshold concentrations (DCVC >2.5 nmol/plate; DCVCS >1000 nmol/plate) (Figure 2). However, TCVC and TCVCS did not appear to induce toxicity in TA100; no microcolony formation or decreasing total number of revertants was observed as TCVC or TCVCS concentration was increased.

Previously, it was demonstrated that in other tester strains (i.e. TA2638), DCVC mutagenicity was reduced in the presence of the β-lyase inhibitor AOAA (Dekant et al., 1986). DCVC is known to be bioactivated by β-lyases, whereas DCVCS is not. Addition of AOAA did not appear to reduce DCVC mutagenicity in TA100 (Figure 4). However, DCVC was mutagenic at higher non-toxic doses in TA2638 (Dekant et al., 1986), whereas DCVC toxicity in TA100 limited the DCVC concentration range that could be tested which rendered it difficult to ascertain the effects of AOAA on DCVC mutagenicity in TA100. The reason for the differences in TA100 and TA2638 susceptibility to DCVC toxicity remains unclear.

## 4. Discussion

DCVCS and TCVCS were definite, but weak, mutagens in the TA100 tester strain for the Ames mutagenicity assay. Interestingly, the two reactive sulfoxides differed in their effects in the TA100 strain. TCVCS (Figure 3B) had 3-fold higher mutagenic activity than DCVCS (Figure 2B). In addition, DCVCS appeared to induce toxicity based on observations of increased microcolony formation and decreased numbers of revertants at concentrations above 1000 nmol/plate whereas no toxicity was observed in TA100 exposed to TCVCS. The differences between DCVCS and TCVCS mutagenicity/toxicity in TA100 may be related to the differences in the stability of the compounds in the presence of thiols. DCVCS stability in the presence of GSH ($t_{1/2}$ of 1.2 min) is significantly lower than TCVCS stability ($t_{1/2}$ of 20 min) (Ripp et al., 1997). It is possible that the lower stability of DCVCS results in rapid formation of protein adducts and induction of toxicity, whereas the higher stability of TCVCS may allow for more selectivity toward targets associated with mutagenic effects.

Both DCVCS and TCVCS were less mutagenic than their respective precursor cysteine *S*-conjugates, DCVC (Figure 2A) and TCVC (Figure 3A). Similar to DCVCS, DCVC appeared to induce toxicity in the bacteria above threshold concentrations (> 2.5 nmol/plate), limiting the concentration range that could be tested for these compounds with this strain. On the other hand, TCVC and TCVCS did not appear to induce toxicity at the concentrations tested.

The reason for the comparatively lower mutagenicity of DCVCS and TCVCS compared to DCVC and TCVC may be related to the selective reactivity of DCVCS and TCVCS toward sulfhydryl groups. DCVCS will form adducts with *N*-acetyl cysteine but not *N*-acetyl lysine or L-valinamide (Barshteyn and Elfarra, 2007). The β-lyase-derived reactive metabolites generated from DCVC and TCVC have the ability to react with other nucleophilic sites,

such as the ε-amino group of lysine (Birner et al., 1994; Pähler et al., 1999b; Barshteyn and Elfarra, 2009). DCVCS and TCVCS selectivity toward sulfhydryl groups may indicate a preference for forming adducts with proteins rather than nucleic acids. On the other hand, the β-lyase-derived reactive metabolites can target nucleophilic nitrogen atoms found in both nucleic acids (e.g. amino groups and nitrogen atoms in the aromatic rings) and proteins (e.g. amino groups), suggesting that they may play a more significant role in the mutagenicity of DCVC and TCVC.

The observed toxicity of the metabolites in TA100 appear to run counter to the observation that DCVCS and TCVCS are more potent nephrotoxicants (and cytotoxicants) than DCVC and TCVC in mammalian kidney or proximal tubular cells and that TCVC may be a more potent nephrotoxin and cytotoxicant than DCVC. However, differences between mammalian model systems used in previous studies and the bacterial system used in the present study may explain why the results are not parallel between the current studies and previously reported mammalian toxicity data. For example, β-lyase activity in TA100 is 3-fold higher compared to rat kidney cytosol, suggesting that differences in metabolism may, in part, explain the differences in observed toxicities in these model systems.

Surprisingly, AOAA treatment did not appear to have a significant effect on the mutagenic effects of DCVC in the TA100 tester strain. However, TA100 is known to be more sensitive to the mutagenic effects of TCVC and less sensitive for DCVC (Dekant et al., 1986). In addition, the toxicity of DCVC in this strain may, in part, also limit the ability to observe alteration of DCVC mutagenicity upon treatment with AOAA. Furthermore, the innate toxicity of AOAA limited the usable concentration of AOAA. TA100 has been shown to have high β-lyase expression (Dekant et al., 1986). Therefore, it is possible that the AOAA concentration used was not sufficient to fully inhibit β-lyase-dependent bioactivation of DCVC.

Although the mutagenicity assay results suggest that DCVCS and TCVCS may not play a significant role in the mutagenicity of DCVC and TCVC in the TA100 strain, additional studies using other strains should be carried out before further conclusions on the role of the sulfoxides in TCE and PCE mutagenicity can be made. The additional use of an alternative strain, such as TA2638, which has been shown to be more sensitive to the mutagenic effects of DCVC and less sensitive for TCVC (Dekant et al., 1986), should be considered.

Other potentially relevant factors should be considered before making further conclusions on the role of sulfoxidation in TCE and PCE mutagenicity. Differences between *S. typhimurium* tester strains could be factors in determining sensitivity to mutagenic activity and toxicity of the metabolites. Tester strains can differ in bioactivation enzyme activity levels. For example, β-lyase activity in TA2638 is 1.75-fold higher than TA100 (Dekant et al., 1986). Another potential factor that should be considered is that *S. typhimurium* strains are also known to contain and export variable amounts of GSH into the extracellular medium (Owens and Hartman, 1986). Since DCVCS is known to react with sulfhydryl-containing macromolecules, including GSH (Sausen and Elfarra, 1991), the GSH levels could directly affect the effective DCVCS concentration reaching the bacteria and therefore variations in GSH content between strains could play a significant role in determining the apparent mutagenicity of the compound.

## 5. Conclusions

Collectively, the results from the present study suggest that bioactivation of the cysteine *S*-conjugates DCVC and TCVC via sulfoxidation does not play a significant role in their mutagenicity in the Ames TA100 tester strain mutagenicity assay. Significantly more DCVCS or TCVCS is required to achieve similar levels of revertants as the parent

compounds. However, further studies with alternative tester strains are needed to confirm these results and allow conclusions to be made regarding the role of sulfoxidation in cysteine *S*-conjugate mutagenicity.

## Acknowledgments

This work was supported by the National Institutes of Health [R01 DK044295] and the National Institute of Environmental Health Sciences [T32 ES007015].

## Abbreviations

| | |
|---|---|
| **β-lyase** | cysteine *S*-conjugate β-lyase |
| **AOAA** | aminooxyacetic acid |
| **DCVC** | *S*-(1,2-dichlorovinyl)-L-cysteine |
| **DCVCS** | *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide |
| **FMO** | flavin-containing monooxygenase |
| **GSH** | glutathione |
| **PCE** | tetrachloroethylene |
| **TA100** | *Salmonella typhimurium* TA100 tester strain |
| **TA2638** | *Salmonella typhimurium* TA2638 tester strain |
| **TCE** | trichloroethylene |
| **TCVC** | *S*-(1,2,2-trichlorovinyl)-L-cysteine |
| **TCVCS** | *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide |

## References

Barshteyn N, Elfarra AA. Formation of three *N*-acetyl-L-cysteine monoadducts and one diadduct by the reaction of *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide with *N*-acetyl-L-cysteine at physiological conditions: chemical mechanisms and toxicological implications. Chem Res Toxicol. 2007; 20:1563–1569. [PubMed: 17892265]

Barshteyn N, Elfarra AA. Globin monoadducts and cross-links provide evidence for the presence of *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide, chlorothioketene, and 2-chlorothionoacetyl chloride in the circulation in rats administered *S*-(1,2-dichlorovinyl)-L-cysteine. Chem Res Toxicol. 2009; 22:1629–1638. [PubMed: 19694420]

Bernauer U, Birner G, Dekant W, Henschler D. Biotransformation of trichloroethene: dose-dependent excretion of 2,2,2-trichloro-metabolites and mercapturic acids in rats and humans after inhalation. Arch Toxicol. 1996; 70:338–346. [PubMed: 8975632]

Birner G, Richling C, Henschler D, Anders MW, Dekant W. Metabolism of tetrachloroethene in rats: Identification of $N^{\varepsilon}$-(dichloroacetyl)-L-lysine and *N*-(trichloroacetyl)-L-lysine as protein adducts. Chem Res Toxicol. 1994; 7:724–732. [PubMed: 7696525]

Birner G, Rutkowska A, Dekant W. *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine and 2,2,2-trichloroethanol: two novel metabolites of tetrachloroethene in humans after occupational exposure. Drug Metab Dispos. 1996; 24:41–48. [PubMed: 8825189]

Brüning T, Golka K, Makropoulos V, Bolt HM. Preexistence of chronic tubular damage in cases of renal cell cancer after long and high exposure to trichloroethylene. Arch Toxicol. 1996; 70:259–260. [PubMed: 8825688]

Brüning T, Mann H, Melzer H, Sundberg AG, Bolt HM. Pathological excretion patterns of urinary proteins in renal cell cancer patients exposed to trichloroethylene. Occup Med (Lond). 1999; 49:299–305. [PubMed: 10628055]

Brüning T, Vamvakas S, Makropoulos V, Birner G. Acute intoxication with trichloroethylene: clinical symptoms, toxicokinetics, metabolism, and development of biochemical parameters for renal damage. Toxicol Sci. 1998; 41:157–165. [PubMed: 9520351]

Dekant W, Berthold K, Vamvakas S, Henschler D, Anders MW. 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. 1988; 1:175–178. [PubMed: 2979728]

Dekant W, Martens G, Vamvakas S, Metzler M, Henschler D. Bioactivation of tetrachloroethylene: role of glutathione *S*-transferase-catalyzed conjugation versus cytochrome P-450-dependent phospholipid alkylation. Drug Metab Dispos. 1987; 15:702–709. [PubMed: 2891489]

Dekant W, Vamvakas S, Berthold K, Schmidt S, Wild D, Henschler D. Bacterial beta-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene and hexachlorobutadiene. Chem Biol Interact. 1986; 60:31–45. [PubMed: 3536138]

Dekant W, Vamvakas S, Anders MW. Formation and fate of nephrotoxic and cytotoxic glutathione *S*-conjugates: cysteine conjugate beta-lyase pathway. Adv Pharmacol. 1994; 27:115–162. [PubMed: 8068551]

Elfarra AA, Krause RJ. *S*-(1,2,2-Trichlorovinyl)-L-cysteine sulfoxide, a reactive metabolite of *S*-(1,2,2-trichlorovinyl)-L-cysteine formed in rat liver and kidney microsomes, is a potent nephrotoxicant. J Pharmacol Exp Ther. 2007; 321:1095–1101. [PubMed: 17347324]

Elfarra AA, Jakobson I, Anders MW. Mechanism of *S*-(1,2-dichlorovinyl)glutathione-induced nephrotoxicity. Biochem Pharmacol. 1986; 35:283–288. [PubMed: 2867768]

Jha AM, Singh AC, Sinha U, Kumar M. Genotoxicity of crotonaldehyde in the bone marrow and germ cells of laboratory mice. Mutat Res. 2007; 632:69–77. [PubMed: 17543575]

Krause RJ, Lash LH, Elfarra AA. Human kidney flavin-containing monooxygenases and their potential roles in cysteine *S*-conjugate metabolism and nephrotoxicity. J Pharmacol Exp Ther. 2003; 304:185–191. [PubMed: 12490590]

Lash LH, Anders MW. Cytotoxicity of *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-L-cysteine in isolated rat kidney cells. J Biol Chem. 1986; 261:13076–13081. [PubMed: 2875994]

Lash LH, Parker JC. Hepatic and renal toxicities associated with perchloroethylene. Pharmacol Rev. 2001; 53:177–208. [PubMed: 11356983]

Lash LH, Fisher JW, Lipscomb JC, Parker JC. Metabolism of trichloroethylene. Environ Health Perspect. 2000; 108(Suppl 2):177–200. [PubMed: 10807551]

Lash LH, Qian W, Putt DA, Desai K, Elfarra AA, Sicuri AR, Parker JC. Glutathione conjugation of perchloroethylene in rats and mice in vitro: sex-, species-, and tissue-dependent differences. Toxicol Appl Pharmacol. 1998; 150:49–57. [PubMed: 9630452]

Lash LH, Sausen PJ, Duescher RJ, Cooley AJ, Elfarra AA. Roles of cysteine conjugate beta-lyase and *S*-oxidase in nephrotoxicity: studies with *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide. J Pharmacol Exp Ther. 1994; 269:374–383. [PubMed: 8169843]

Mortelmans K, Zeiger E. The Ames salmonella/microsome mutagenicity assay. Mutat Res. 2000; 455:29–60. [PubMed: 11113466]

National Toxicology Program. NTP 12th Report on Carcinogens. Rep Carcinog. 2011; 12:iii-499.

Owens RA, Hartman PE. Export of glutathione by some widely used Salmonella typhimurium and Escherichia coli strains. J Bacteriol. 1986; 168:109–114. [PubMed: 3531162]

Pähler A, Parker J, Dekant W. Dose-dependent protein adduct formation in kidney, liver, and blood of rats and in human blood after perchloroethene inhalation. Toxicol Sci. 1999; 48:5–13. [PubMed: 10330678]

Pähler A, Volkel W, Dekant W. Quantitation of $N^{\varepsilon}$-(dichloroacetyl)-L-lysine in proteins after perchloroethene exposure by gas chromatography-mass spectrometry using chemical ionization and negative ion detection following immunoaffinity chromatography. J Chromatogr A. 1999; 847:25–34. [PubMed: 10431349]

Parent RA, Caravello HE, San RH. Mutagenic activity of acrolein in S. typhimurium and E. coli. J Appl Toxicol. 1996; 16:103–108. [PubMed: 8935782]

Ripp SL, Overby LH, Philpot RM, Elfarra AA. 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. 1997; 51:507–515. [PubMed: 9058607]

Sausen PJ, Elfarra AA. Reactivity of cysteine *S*-conjugate sulfoxides: formation of *S*-[1-chloro-2-(*S*-glutathionyl)vinyl]-L-cysteine sulfoxide by the reaction of *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide with glutathione. Chem Res Toxicol. 1991; 4:655–660. [PubMed: 1807449]

Vamvakas S, Kordowich FJ, Dekant W, Neudecker T, Henschler D. Mutagenicity of hexachloro-1,3-butadiene and its *S*-conjugates in the Ames test role of activation by the mercapturic acid pathway in its nephrocarcinogenicity. Carcinogenesis. 1988; 9:907–910. [PubMed: 3286031]

Vamvakas S, Herkenhoff M, Dekant W, Henschler D. Mutagenicity of tetrachloroethene in the Ames test--metabolic activation by conjugation with glutathione. J Biochem Toxicol. 1989; 4:21–27. [PubMed: 2671372]

Vamvakas S, Köchling A, Berthold K, Dekant W. Cytotoxicity of cysteine *S*-conjugates: structure-activity relationships. Chem Biol Interact. 1989; 71:79–90. [PubMed: 2776234]

Vermeulen R, Zhang L, Spierenburg A, Tang X, Bonventre JV, Reiss B, Shen M, Smith MT, Qiu C, Ge Y, Ji Z, Xiong J, He J, Hao Z, Liu S, Xie Y, Yue F, Guo W, Purdue M, Beane Freeman LE, Sabbisetti V, Li L, Huang H, Rothman N, Lan Q. Elevated urinary levels of kidney injury molecule-1 among Chinese factory workers exposed to trichloroethylene. Carcinogenesis. 2012; 33:1538–1541. [PubMed: 22665366]

Völkel W, Friedewald M, Lederer E, Pähler A, Parker J, Dekant W. 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. 1998; 153:20–27. [PubMed: 9875296]

## Highlights

- DCVCS and TCVCS mutagenicity investigated in the Ames *S. typhimurium* TA100 strain.
- DCVCS and TCVCS were definite but weak mutagens in TA100.
- DCVCS and TCVCS were less mutagenic than the precursors DCVC and TCVC.
- DCVC and TCVC metabolite reactivity may explain differences in mutagenicity.

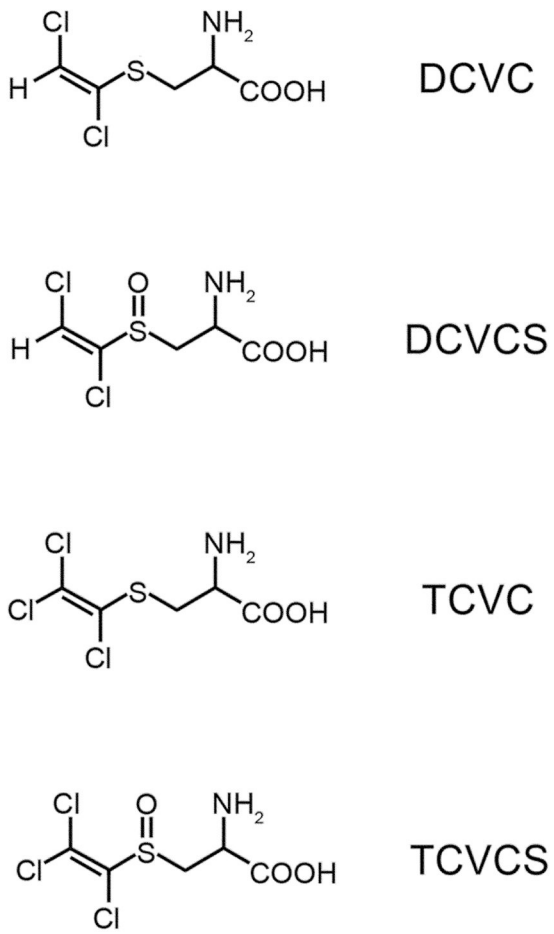


**Figure 1.**
Structures of *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (DCVCS), *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), and *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVCS).

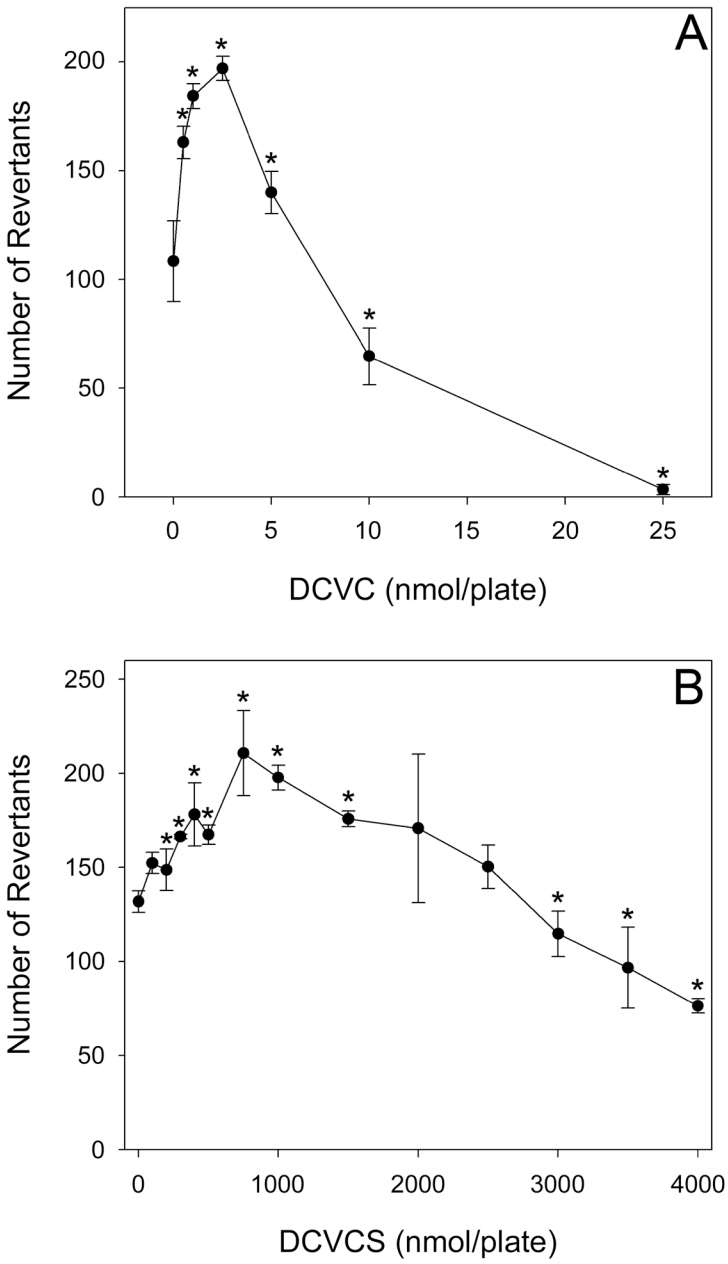


**Figure 2.**
Cultures of *S. typhimurium* TA100 were exposed to various concentrations of DCVC (A) and DCVCS (B) and mutagenicity assayed by the Ames Salmonella mutagenicity assay. Graphs depict the number of revertants induced as test chemical concentration is increased. Points and bars represent means ± SD of triplicates at each test chemical concentration. * $p < 0.05$ compared to the spontaneous reversion rate (TA100 exposed to buffer alone).

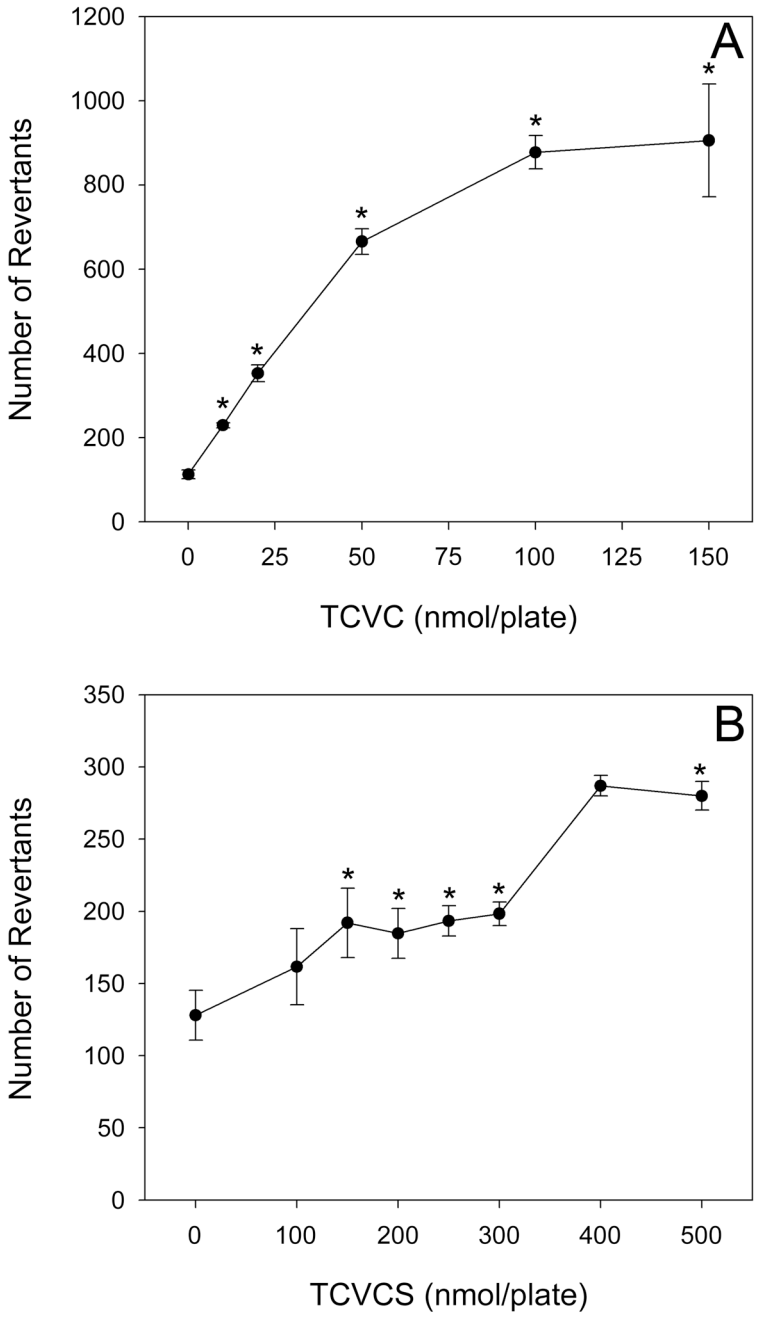


**Figure 3.**

Cultures of *S. typhimurium* TA100 were exposed to various concentrations of TCVC (A) and TCVCS (B) and mutagenicity assayed by the Ames Salmonella mutagenicity assay. Graphs depict the number of revertants induced as test chemical concentration is increased. Points and bars represent mean ± SD of triplicates at each test chemical concentration (except TA100 at 400 nmol/plate TCVCS which is in duplicate). *$p < 0.05$ compared to the spontaneous reversion rate (TA100 exposed to buffer alone).

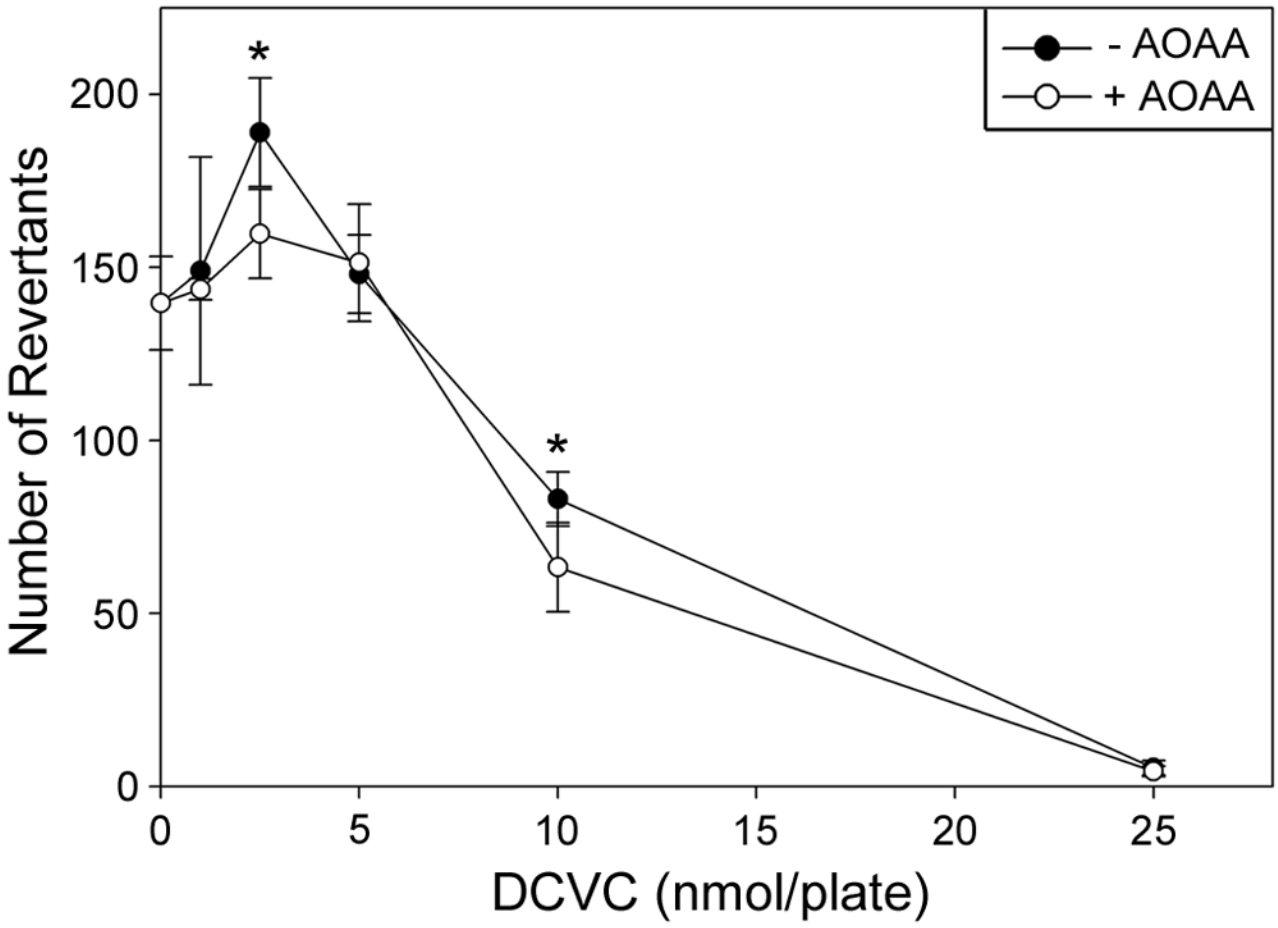


**Figure 4.**

Comparison of cultures of *S. typhimurium* TA100 incubated with various concentrations of DCVC alone (●) or in the presence of 1 mM AOAA (○). Points and bars represent mean ± SD of triplicates at each test chemical concentration. *$p < 0.05$ when comparing revertants/nmol DCVC between TA100 ± AOAA at the same concentration of DCVC.