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# Chromatographic and Mutagenic Analyses of 1,2-Dichloropropane and 1,3-Dichloropropylene and Their Degradation Products

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It was the intent of this study to analyze for the suspected carcinogens resulting from the use of the soil fumigant Vorlex in selected Connecticut potable water (Connors et al. 1988) and to evaluate the mutagenic activity of the two known hydrolysis/oxidative products, 3-chloroallyl alcohol (3-CAA) and 3-chloropropenoic acid (3-CPA). This information is useful for state and local environmental health officials in determining whether or not residents should be required to drink bottled water. 3-CAA and 3-CPA tested positive in the Ames/Salmonella typhimurium mutagenicity test with metabolic activation by PCB induced SA9 rat liver enzyme. However, no 3-CAA was found in any of the potable water samples we tested. Every sample did have a measurable level of the Vorlex biocide, 1,2-dichloropropane.

The extensive use of organohalogen soil fumigants has resulted in the contamination of some Connecticut potable water supplies. The pesticide Vorlex, first registered in Connecticut in the mid 1960's, was extensively used by farmers as a soil fumigant (up to 50 gallons per acre per year) to control nematodes and soil-borne plant diseases, primarily on tobacco, nursery stock, certain vegetables, and strawberries. After 1983, the Vorlex formulation changed from predominantly 1,2-dichloropropane (1,2-DCP) to 1,3-dichloropropylene (1,3-DCP) (for a detailed discussion of 1,2-DCP and 1,3-DCP groundwater contamination, see Cohen (1983) and for health affects, see Thomas (1986). We suspect that 1,2-DCP persists in soil much like 1,2-dibromoethane or ethylene dibromide (EDB) has been reported to do (Pignatello et al. 1987).

This study evolved because there is a problem with monitoring for 1,3-DCP in moist soils. It is readily, nonbiologically, hydrolyzed (nucleophilic substitution) to 3-chloro-2-propen-1-ol (3-chloroallyl alcohol) (Castro + Belser 1966) which is a potent human skin irritant with known fungicidal, nematodicidal, and phytotoxic properties (Baines et al. 1977). To complicate monitoring further, 3-CAA is biologically oxidized to 3-chloropropenoic acid (3-CPA), which is oxidized to formylacetic acid (FAA) which eventually decarboxylates to carbon dioxide, see Figure I.

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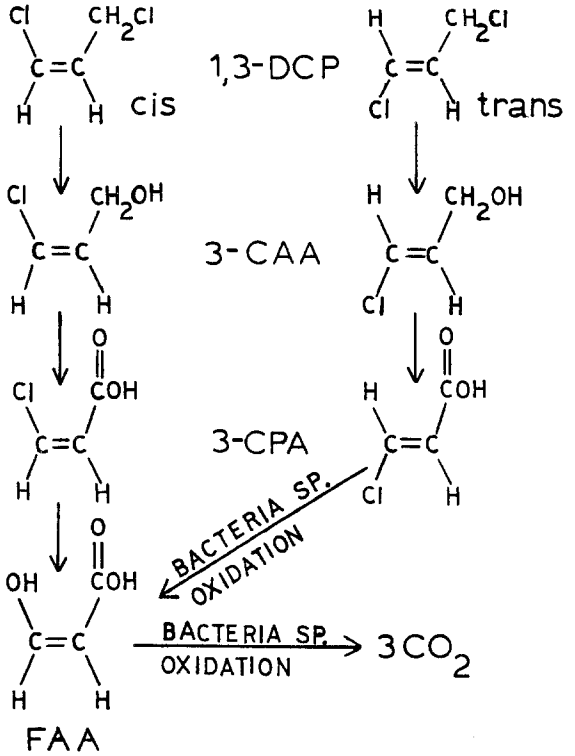


Figure I. Degradation of cis- and trans-1,3-DCP.

Thus, persistence of 3-CAA and 3-CPA in soil is dependent on the type of microbes present (Belser + Castro 1971), temperature (Van Dijk 1974), and soil-type (Roberts + Stoydin 1976). Currently, the Connecticut Department of Health Services Laboratory uses EPA method 501.2 to analyze potable water for 1,3-DCP. However, 1,3-DCP is rarely detected and it's hydrolysis/oxidative products are not amenable to analysis by this EPA method.

The immediate health hazards of 3-CAA and 3-CPA are unclear. However, Talcott + King (1984) reported that 1,3-DCP and its polar impurities such as epichlohydrin (1-chloro-2,3 epoxypropane) and 1,3-dichloro-2-propanol were mutagenic in the Ames test. Thus, we felt that because the mutagenicities of 3-CAA and 3-CPA had not been reported (Wassom JS (1987), Director, Environmental Mutagen, Carcinogen and Teratogen Information Program, Oak Ridge National laboratory, Oak Ridge, TN, personal communication, May 7), they should be determined.

## MATERIALS AND METHODS

1,2-DCP and cis- and trans-1,3-DCP were obtained from Chem Service, Inc., in >99% purity; cis- and trans-3-CAA were obtained from Dow Chemical Co., Agricultural Products Dept., Midland, Michigan in 96.0 and 96.5% purity, respectively, and stored at -5°C. The cis- and trans-3-CPA, 98 and 99% purity respectively, were obtained from Aldrich Chemical Co. All compounds were used as received. Cis- and trans-3-CAA showed no signs of degradation after a six-month storage period; analysis was by gas chromatography/mass spectrometry.

For EPA method 501.2 (pentane extraction, GC/EC), gas chromatography of 1,2-DCP and 1,3-DCP was done with a Perkin Elmer 3920B gas chromatograph equipped with an electron-capture detector. The borosilicate glass column was 1.8 m x 4 mm i.d. and was packed with 10% Squalane on 80/100 mesh Chromosorb WSW; a 67°C oven temp., 275°C detector temp., 180°C inlet temp., and a nitrogen carrier gas at a flow rate of 62 mL/min were used. For analysis of 3-CAA, a method developed by Dow Chemical Co. (ether/hexane extraction, GC) (Bjerke 1987) was used with a Sigma 300 gas chromatograph equipped with an electron-capture detector. The borosilicate glass column was 1.8 m x 4 mm i.d. and was packed with 10% SP-1000 on 100/120 mesh Chromosorb WAW; a 135°C oven temp., 300°C detector temp., 150°C inlet temp. and a nitrogen carrier gas at a flow rate of 65 mL/min were used. For confirmatory analyses on samples showing positive 1,2-DCP residues, EPA Method 501.1 (purge and trap, widebore Vocol column, Hall Conductivity detector) and EPA method 524.1 (purge and trap, 1% SP-1000, MS detector) were used.

For mutagenicity tests, diagnostic mutagens, sodium azide and 2-aminofluorene were obtained from Aldrich Chemical Co. and used as received. Salmonella typhimurium bacterial strains were obtained from Bruce Ames Laboratory, University of California, Berkeley, CA and used to quantify the mutagenic activity of cis- and trans-3-CAA and cis- and trans-3-CPA. Strains TA100, 97, and 102 were selected because previous studies (Cohen 1983) showed them to be most sensitive to dichloropropane mutagenisis. PCB induced S9 rat liver enzyme was obtained from Litton Bionetics of Charleston, SC in frozen 5 mL aliquots. β-Nicotinamide adenine dinucleotide phosphate (NADP) and D-glucose-6-phosphate monosodium salt were obtained from Sigma Chemical Co. and used as received. Salt and sodium phosphate buffer solutions were prepared with sterile distilled water. Unless otherwise noted, a high S9 mix was used in all tests. Linear regression analyses of mutagenicity data were done on a Wang microcomputer system. Mutagenicity tests were performed with and without metabolic activation by PCB induced SA9 rat liver enzyme following procedures (plate incorporation method) described by Maron + Ames (1983).

## RESULTS AND DISCUSSION

EPA Method 501.2 was used to analyze potable water samples from

three Connecticut communities where homeowners were reported to have levels of 1,2-DCP greater than the Connecticut Department of Environmental Protection's action level of 10 µg/L (ppb). 1,2-DCP eluted at 3.7 min, cis-1,3-DCP at 4.4 min, and trans-1,3-DCP at 5.3 min. This method was not amenable to the extraction and separation of the hydrolysis/oxidative products of 1,3-DCP. To determine whether or not 1,3-DCP could be readily hydrolyzed to 3-CAA under laboratory conditions, a 1.0 ppb solution of 1,3-DCP was prepared in pH 7.0 buffer. The solution was allowed to stand at ambient room temperature, and the disappearance of 1,3-DCP was followed over time using method 501.2. Assuming pseudo first-order hydrolysis rate constants, half-lives were calculated. These results were compared to values reported by Van Dijk (1974); see Table 1. The values obtained by the present work were within the range of values reported by Van Dijk (1974). Thus this work confirms the rapid degradation of 1,3-DCP under normal environmental conditions.

Table 1. Comparison of pseudo first-order rate constants and half lives for the disappearance of 1,3-DCP in buffered water.

| | Cis-1,3 DCP | | | Trans-1,3-DCP | | |
|---|---|---|---|---|---|---|
| temp. (°C) | 15°C[a] | 25°C[b] | 29°C[a] | 15°C[a] | 25°C[b] | 29°C[a] |
| pH | 5.5 | 7.0 | 5.5 | 5.5 | 7.0 | 5.5 |
| k (days $^{-1}$) | 0.06 | 0.15 | 0.36 | 0.05 | 0.15 | 0.37 |
| $t_{1/2}$ (days) | 11.0 | 4.6 | 2.0 | 13.0 | 4.6 | 2.0 |

[a]Van Dijk (1974).
[b]Present work.

A method adopted from Dow Chemical Co. (Bjerke 1987) was used for the extraction of 3-CAA from potable water samples. It involved saturating a 50-mL sample with NaCl, doing two 25-mL extractions with diethyl ether, adding 2 mL of hexane, and using a three-ball Snyder column to slowly vaporize the diethyl ether and concentrating the 3-CAA to a final volume of 2 mL. To test the method, a 50-mL water sample was fortified with 100 ppb each of cis- and trans-3-CAA. Under the chromatographic conditions described, cis-3-CAA eluted at 7.4 min and trans-3-CAA at 10.6 min. Recoveries were 93.3 and 92.5% for cis- and trans-3-CAA, respectively. However, the chromatographic analysis of 3-CPA was impossible using either method 501.2 or the Dow method. Table 2 summarizes the chromatographic analyses of selected Connecticut potable water samples. The results for 3-CAA are consistent with those reported by Maddy et al. (1982). They too, did not find 3-CAA in a study of well water in selected California communities. The GC/MS chromatogram (from EPA Method 524.1) for the positive Bolton sample, homeowner no. 5, showed Vorlex (as 1,2-DCP) at the 19 µg/L level. (This homeowner would be required to drink bottled water until the level of 1,2-DCP dropped below 10 µg/L.)

Table 2. Analysis of Selected Connecticut Potable Water Samples for Vorlex Biocides. Samples were collected between Sept-Nov,1987.

| Town/Community | Homeowner No. | 1,2-DCP[a] (µg/L) | 1,3-DCP[a,b] | 3-CAA[c,d] |
|---|---|---|---|---|
| East Windsor | 1 | 2.2[e] | ND | ND |
| | 2 | 2.8 | ND | ND |
| Suffield | 3 | 1.7[e] | ND | ND |
| | 4 | 2.0 | ND | ND |
| Bolton | 5 | 19.0[f,g] | ND | ND |
| | 6 | 0.7 | ND | ND |
| | 7 | 0.8 | ND | ND |
| | 8 | 7.4[f] | ND | ND |

[a]EPA Method 501.2 [b]None detected at LOD = 0.10 µg/L at three X S/N [c]Dow adopted Method [d]None detected at LOD = 0.25 µg/L at three X S/N [e]Verified by EPA Method 501.1 [f]Verified by EPA Method 524.1 [g]Above Conn. Dept. Envrn. Prot. Action Level of 10 µg/L

The mutagenic activities of 1,2-DCP and 1,3-DCP have been reported (Cohen 1983). We tested the mutagenic activity of cis- and trans-3-CAA with strain TA100 with and without SA9 activation. No mutagenicity was observed without SA9 (the number of revertants per plate over a range of amounts, 0.01 to 1000 µg, were never higher than the solvent control). However, with SA9 (high concentration) the mutagenic activity of cis-3-CAA was found to be approximately $5.04 \times 10^5$ rev/mg (mutagenic activity reported as the slope from linear regression analysis in the nontoxic region of the dose-response plot), on the same order of magnitude as that determined by Talcott + King (1984) for a high-boiling dichloropropene preparation ($5.49 \times 10^5$ rev/mg). The trans compound was also mutagenic, $4.32 \times 10^4$, but not as active as cis-3-CAA. The positive control in these tests was 2-aminofluorene at 20 µg/plate which gave approximately 1000 net rev/plate and the solvent control was dimethylsulfoxide (DMSO) which gave a spontaneous rate of 200 to 230 rev/plate. Investigation of the mutagenic mode of action of these chemicals is beyond the scope of this paper. However, there have been reports that for 2- and 3-carbon halogenated compounds, an alcohol group and the position of a double bond in relation to the halogen may influence mutagenic activity (Stolzenberg + Hine 1980); this could explain the different observed mutagenic activities of cis- and trans-3-CAA. We also tested the mutagenic activity of cis- and trans-3-CPA using strains 97 and 102 with and without activation by SA9. They were both mutagenic to these strains with SA9 in the nontoxic range of <10 µg per plate and the number of rev/plate were two to three times above the solvent control of 282 rev/plate.

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REFERENCES

Baines RC, Klotz LJ, DeWolfe TA (1977) Some Biocidal Properties of 1,3-DCP and its Degradation Product. Phytopathology 67:936-40

Belser NO, Castro CE (1971) Biodehalogenation-The Metabolism of the Nematocides cis- and trans-3-chloroallyl Alcohol by a Bacterium Isolated from Soil. J Agric Food Chem 19:23-26

Bjerke EL (1987) Agric Chemistry R and D Labs. Agricultural Products Dept., Dow Chemical Co., Midland, MI 48640, personal communication Feb. 3

Castro CE, Belser NO (1966) Hydrolysis of Cis- and Trans-1,3-Dichloropropene in Wet Soil. J Agric Food Chem 14:69-70

Cohen DB (1983) 1,2-Dichloropropane and 1,3-Dichloropropene. Specials Projects Report No. 83-8sp, California State Water Resources Control Board, Toxic Substances Control Program, Sacramento, CA

Connors, TF, Pita AM, Michaud RA, Carlone SE (1988) Analysis of Organohalide-Biocide Hydrolysis Products in Connecticut Potable Water. The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Abstract No. 181, New Orleans, LA, February 22-26

Maddy KT, Fong HR, Lowe JA, Conrad DW, Fredrickson AS (1982) A Study of Well Water in Selected California Communities for Residues of 1,3-Dichloropropene, Chloroallyl Alcohol and 49 Organophosphate or Chlorinated Hydrocarbon Pesticides. Bull Environ Contam Toxicol 29:354-359

Maron DM, Ames BN (1983) Revised Methods for the Salmonella Mutagenicity Test. Mutation Research 113:173-215

Pignatello JJ, Shawney BL, Frink CR (1987) EDB Persistence in Soil. Science 236:898

Roberts TR, Stoydin G (1976) The Degradation of (Z)- and (E)-1,3- Dichloropropenes and 1,2-Dichloropropane in Soil. Pestic Sci 7:325-335

Stolzenberg SJ, Hine CH (1980) Mutagenicity of 2- and 3-Carbon Halogenated Compounds in the Salmonella/Mammalian-Microsome Test. Environmental Mutagenesis 2:59-66

Talcott RE, King J (1984) Mutagenic Impurities in 1,3-Dichloropropene Preparations. National Cancer Institute 72:1113-1116

Thomas RD (Ed) (1986) Drinking Water and Health. Vol. 6, National Academy Press, Washington D.C., pp 326-337

Van Dijk H (1974) Degradation of 1,3-Dichloropropenes in the Soil. Agro-Ecosystems 1:193-204

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