Fate of 2,2,2-Trichloroacetaldehyde (Chloral Hydrate) Produced during Trichloroethylene Oxidation by Methanotrophs

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Four different methanotrophs expressing soluble methane monooxygenase produced 2,2,2-trichloroacetaldehyde, or chloral hydrate, a controlled substance, during the oxidation of trichloroethylene. Chloral hydrate concentrations decreased in these cultures between 1 h and 24 h of incubation. Chloral hydrate was shown to be biologically transformed to trichloroethanol and trichloroacetic acid by Methylosinus trichosporium OB3b. At elevated pH and temperature, chloral hydrate readily decomposed and chloroform and formic acid were detected as products.

Trichloroethylene (TCE) is a widespread groundwater pollutant (31). Its prevalence as an industrial solvent is due in part to its relatively low toxicity. For example, the 50% lethal dose of TCE in rats is 7,200 mg per kg of body weight (29). Concern over TCE contamination of waters and soils stems from observations that some anaerobic bacteria biotransform TCE with the subsequent formation of vinyl chloride (34), a potent mutagen and carcinogen (23, 28). This has prompted research on the use of aerobic bacteria which biosynthesize nonspecific catabolic oxygenases capable of cooxidizing TCE (3, 10, 16, 27, 32, 35, 36, 39). Soluble methane monooxygenase (sMMO), biosynthesized by certain methanotrophs, oxidizes TCE in vitro at a rate of 682 nmol/min per mg of protein (11). In vivo, TCE and vinyl chloride are oxidized most rapidly by bacteria expressing sMMO (5, 11, 26). For this reason, there is great interest in using methanotrophs for bioremediation of low-molecular-weight halogenated solvents (15).

In the course of degrading chlorinated compounds, oxygenases can produce reactive or toxic intermediates (1, 18, 37). In this context, the products of TCE oxidation were determined by using sMMO purified from Methylosinus trichosporium OB3b (11). TCE epoxide is the major oxygenated intermediate formed, and it rapidly undergoes spontaneous decomposition in aqueous media. A stable side-product of TCE oxygenation, 2,2,2-trichloroacetaldehyde, accounts for 6% of the total TCE consumed. This minor product is also known as chloral hydrate due to the preponderance of the hydrated form of the aldehyde in aqueous media (24). Chloral hydrate is a controlled substance. When used in conjunction with alcoholic beverages, it is known as "knock-out drops" or "Mickey Finns" (6). The 50% lethal dose of chloral hydrate in rats is 479 mg per kg of body weight, which is 15 times lower than the 50% lethal dose of TCE (12). Chloral hydrate is a mutagen, and it is known to cause chromosomal damage, or aneuploidy (4, 9, 14, 17, 21, 33). Thus, proposed bioremediation schemes using methanotrophic bacteria must take into account the formation and fate of this potentially toxic metabolite. Chloral hydrate has been reported to be a TCE oxidation product only for M. trichosporium OB3b (27); its possible metabolism by methanotrophs has not been previously investigated. In the present study, three other methanotrophs were shown to produce chloral hydrate. Additionally, the transformation of chloral hydrate via biological and abiotic routes was demonstrated and the products of those reactions were identified.

Previous studies showed that rapid rates of TCE oxidation by methanotrophs correlate with the expression of sMMO (5, 27, 32). The sMMO of M. trichosporium OB3b produces chloral hydrate as an oxidation product of TCE both in vitro (11) and in vivo (27). In the present study, batch incubation experiments were conducted to determine whether other methanotrophs also produce chloral hydrate from TCE. The methanotrophs were grown in a mineral salts medium (8) with shaking at 200 rpm under a 1:4 (vol/vol) methane-air atmosphere. The medium was not supplemented with copper, except for Methylomonas methanica cultures, to which 1 μM copper sulfate was added. Copper addition is required for the growth of Methylomonas methanica (5). Each culture was grown to late exponential phase, harvested by centrifugation, resuspended to a cell density corresponding to an A₅₀₀ of 1.0 ± 0.1 in mineral salts medium containing 50 mM sodium formate at pH 7.0, and dispensed into crimp-sealed septum vials as previously described (5, 36). TCE was added from a stock solution in water to a final concentration of 13 to 30 μM. Vials containing viable and heat-killed (control) cells were incubated for 1 h and 24 h at 30°C, except for Methylococcus capsulatus, which was incubated at 37°C. Incubation mixtures were extracted with diethyl ether and analyzed for TCE disappearance as well as for chloral hydrate and 2,2,2-trichloroethanol formation by gas chromatography by using previously described methods (11).

As shown in Table 1, all four of the methanotrophs that oxidized TCE at appreciable rates produced chloral hydrate. Heat-killed controls showed less than 10% decrease in TCE, and chloral hydrate was not detected in these cultures. Methylomonas methanica did not oxidize TCE at a detectable rate and did not produce chloral hydrate, and it is reported to biosynthesize only a membrane-bound methane monooxygenase (2). Thus, both rapid TCE oxidation and chloral hydrate formation may be characteristic features of sMMO enzyme systems. In a previous study of TCE oxidation by sMMO from Methylomonas capsulatus, chloral hydrate was not reported as a product (13). However, the detection of chloral hydrate by gas chromatography-flame ionization detection, as described in that report, is much less sensitive than the electron capture detection methods used here. Since chloral hydrate is a minor product of TCE oxidation by sMMO, it may have been formed at levels
Methylosporovibrio #27 (5)

Methylomonas methanica (5)

Methylococcus capsulatus (38)

Methylosinus sporium OB3b (5)

M. trichosporium OB3b (5)

**TABLE 1. TCE oxidation and volatile product formation by methanotrophs**

<table>
<thead>
<tr>
<th>Methanotroph (reference)</th>
<th>Time of incubation</th>
<th>TCE disappearance, nmol/ml (% oxidized)</th>
<th>Volatile products, nmol/ml (% of total products)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloral hydrate</td>
</tr>
<tr>
<td><strong>M. trichosporium OB3b (5)</strong></td>
<td>1 h</td>
<td>13.4 ± 0.4 (98%)</td>
<td>0.60 ± 0.02 (4.6%)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>13.6 ± 0.0 (100%)</td>
<td>0.03 ± 0.00 (0.2%)</td>
</tr>
<tr>
<td><strong>Methylococcus capsulatus (38)</strong></td>
<td>1 h</td>
<td>25.0 ± 11.9 (90%)</td>
<td>0.76 ± 0.06 (2.7%)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>28.4 ± 1.6 (100%)</td>
<td>0.21 ± 0.03 (0.6%)</td>
</tr>
<tr>
<td><strong>Methylosinus sporium #27 (5)</strong></td>
<td>1 h</td>
<td>3.0 ± 0.2 (10%)</td>
<td>0.48 ± 0.01 (16%)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>7.0 ± 0.1 (23%)</td>
<td>0.20 ± 0.00 (2.8%)</td>
</tr>
<tr>
<td><strong>Methylomonas methanica (5)</strong></td>
<td>1 h</td>
<td>16.2 ± 0.7 (85%)</td>
<td>0.81 ± 0.01 (5.0%)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>19.1 ± 0.0 (100%)</td>
<td>0.25 ± 0.05 (1.3%)</td>
</tr>
</tbody>
</table>

*a ND, not detected.*

below analytical detection limits in the previous study. In all of the strains and at the two time points examined, the amount of chloral hydrate detected did not exceed 16% of the TCE oxidized. This is consistent with studies using purified sMMO from *M. trichosporium* OB3b, in which chloral hydrate accounted for 6% of the total products and the remaining TCE was processed through TCE epoxide (11).

Although the percent conversion of TCE to chloral hydrate appeared uniformly low, the greater toxicity of chloral hydrate necessitated an examination of its fate in methanotrophic TCE-oxidizing cultures. All four strains that oxidized TCE showed higher levels of chloral hydrate at 1 h than at 24 h (Table 1). For example, while chloral hydrate accounts for 4.6% of the TCE oxidized by *M. trichosporium* OB3b in 1 h, it is only 0.2% of the TCE consumed after 24 h. There is a concomitant increase in trichloroethanol, suggesting that the methanotroph could catalyze a two-electron reduction of chloral hydrate. Trichloroethanol has previously been observed in *M. trichosporium* OB3b cultures incubated with TCE (27). In other experiments, chloral hydrate was added directly to cultures of *M. trichosporium* OB3b in mineral salts medium without a carbon source to a final concentration of 5 μM. Trichloroethanol was observed to increase with a concomitant decrease in chloral hydrate concentration over 8 h as determined by gas chromatography. However, a decrease in the chloral hydrate concentration of 83 nM per h was matched by an increase in the

![FIG. 1. Transformation of TCE and trichloroacetaldehyde by *M. trichosporium* OB3b and abiotic reactions of the oxidation products.](http://aem.asm.org/)
trichloroethanol concentration of only 40 nM per h. The difference in these reaction stoichiometries was shown not to be due to spontaneous chemical decomposition of chloral hydrate, and these data are presented in the next paragraph.

In an effort to identify a putative metabolic oxidation product(s) of chloral hydrate, \textit{M. trichosporium OB3b} expressing sMMO was incubated with \(^{14}C\)-TCE. The culture fluid was sampled at zero time, 1 h, and 24 h, and a heat-killed control was also sampled. The aliquots were analyzed by high-pressure liquid chromatography by using a Bio-Rad Aminex HPX-87H organic acid column and published methods (11).

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