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# Product Toxicity and Cometabolic Competitive Inhibition Modeling of Chloroform and Trichloroethylene Transformation by Methanotrophic Resting Cells

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**The rate and capacity for chloroform (CF) and trichloroethylene (TCE) transformation by a mixed methanotrophic culture of resting cells (no exogenous energy source) and formate-fed cells were measured. As reported previously for TCE, formate addition resulted in an increased CF transformation rate (0.35 day$^{-1}$ for resting cells and 1.5 day$^{-1}$ for formate-fed cells) and transformation capacity (0.0065 mg of CF per mg of cells for resting cells and 0.015 mg of CF per mg of cells for formate-fed cells), suggesting that depletion of energy stores affects transformation behavior. The observed finite transformation capacity, even with an exogenous energy source, suggests that toxicity was also a factor. CF transformation capacity was significantly lower than that for TCE, suggesting a greater toxicity from CF transformation. The toxicity of CF, TCE, and their transformation products to whole cells was evaluated by comparing the formate oxidation activity of acetylene-treated cells to that of non-acetylene-treated cells with and without prior exposure to CF or TCE. Acetylene arrests the activity of methane monooxygenase in CF and TCE oxidation without halting cell activity toward formate. Significantly diminished formate oxidation by cells exposed to either CF or TCE without acetylene compared with that with acetylene suggests that the solvents themselves were not toxic under the experimental conditions but their transformation products were. The concurrent transformation of CF and TCE by resting cells was measured, and results were compared with predictions from a competitive-inhibition cometabolic transformation model. The reasonable fit between model predictions and experimental observations was supportive of model assumptions.**

Chloroform (CF) and trichloroethylene (TCE) are halogenated organics which are common groundwater contaminants. Both compounds are used as industrial solvents, are volatile, and are transported into the environment by accidental spillage, leaking storage tanks, improper disposal, and landfill leachates. CF is also commonly found in drinking water supplies as a result of chlorination. Both compounds pose a risk when they occur as contaminants in water supplies (50).

CF and TCE are biotransformed under anaerobic conditions to less-chlorinated intermediates and carbon dioxide (4, 7, 8, 21, 22, 34, 36, 51). However, the toxicity, potential carcinogenicity, and persistence of the reaction intermediates make treatment by this approach questionable.

Aerobic biotransformations of CF and TCE in soil columns amended with natural gas demonstrated methanotrophic cometabolism to be a possible biotransformation mechanism (45, 53). Cell-free solutions of methane monooxygenase (MMO), the enzyme responsible for methane oxidation, catalyze the oxidation of CF (15, 38) and TCE (19). Such CF oxidation has been observed with methanotrophic mixed cultures (27, 28) and pure cultures (47), and correspondingly, TCE cometabolism has been observed with methanotrophic mixed cultures (1, 9, 18, 25, 27, 28, 41, 44, 46) and pure cultures (11, 25, 33, 35, 48).

The purposes of this work are to compare the cometabolic transformation rates and extents for CF and TCE by a single mixed methanotrophic culture, to determine the effects of an exogenous energy source (formate) on CF transformation, to measure the transformation capacity for CF, and to use formate oxidation activity to evaluate the potential toxic effects of CF, TCE, and their transformation products to whole cells. Finally, experimental results from the concurrent transformation of CF and TCE are compared with predictions yielded by the incorporation of a competitive-inhibition term into the cometabolic transformation model reported previously (2).

**Background.** CF and TCE were chosen as the targeted compounds in this study because they are related, differing only in the presence of an additional carbon atom and a carbon-carbon double bond in TCE. The mechanism of MMO-catalyzed oxidations is thought to be similar to that of cytochrome P-450-catalyzed oxidations, as both produce the same reactive species of oxygen (19, 20). On this basis, the expected pathway for MMO oxidation of CF is shown in equation 1 (16, 39) (Fig. 1). Similarly, TCE oxidation has been hypothesized to result in formation of epoxide, which can then be chemically and biologically converted to mineralized end products, as shown in equation 2 (19, 33) (Fig. 2).

TCE epoxide is an extremely short-lived, highly reactive molecule (26) that is toxic in mammalian systems (10). Thus the observed toxicity to whole methanotrophic cells during TCE transformation may result from the epoxide or its degradation products rather than from TCE itself. Similarly, the phosgene intermediate anticipated from CF oxidation is an unstable electrophilic compound which may irreversibly bind to cellular proteins and lipids (16, 39, 54), resulting in toxicity to methanotrophic cells.

MMO-catalyzed reactions require oxygen and NADH

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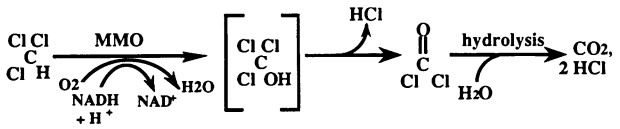


FIG. 1. Equation 1. Expected pathway for MMO oxidation of CF.

(17); thus, cometabolism often requires a supplemental source of NADH (29, 43). NADH can be produced from methane oxidation, endogenous energy reserves, and oxidation of formate, an intermediate of methane oxidation which cannot be used for cell growth (14, 17, 29, 32). Therefore, exogenous formate can be used to replenish the supply of NADH for cometabolic transformations (24, 29, 38), as was shown for methanotrophic TCE cometabolism (1, 35).

TCE transformation by resting methanotrophic cells slows with time and eventually ceases (1, 35, 41, 48), indicating that resting methanotrophic cells have a finite transformation capacity ($T_c$; milligrams of TCE per milligram of cells). This finite $T_c$ has been attributed to diminished reductant supply, toxicity of TCE, and/or toxicity of TCE transformation products (1, 48). Although addition of formate as an exogenous energy source increased $T_c$, the extent of the increase was limited. Additionally, methane consumption was significantly decreased following TCE transformation, suggesting that either TCE itself or its transformation products were exerting toxic effects (1). Although product toxicity was suggested by these studies, the study given here is believed to be more conclusive.

**Model development.** The hypothesized similarity in transformation mechanisms for TCE and CF suggests that the cometabolic transformation model developed previously for TCE (2) could be appropriately applied to CF transformation. Here, the rate of cometabolism is described similarly to Monod kinetics as

$$-dS_i/dt = \frac{k_i X S_i}{K_{si} + S_i} \tag{3}$$

where $S_i$ is the concentration of cometabolized contaminant i at time $t$ (milligrams per liter), $X$ is the active microbial concentration at time $t$ (milligrams per liter), $k_i$ is the maximum rate of contaminant i transformation (milligrams of i per milligram [dry weight] of cells per day), and $K_{si}$ is the half velocity constant for $i$ (milligrams per liter). In a batch reactor with resting cells, the microbial activity ($X$) decreases with time because of utilization of transformation capacity. This effect can be described as follows (2):

$$X = X_o - \frac{1}{T_{ci}}(S_{oi} - S_i) \tag{4}$$

where $X_o$ is the initial active microbial concentration (milligrams per liter), $S_{oi}$ is the initial concentration of cometabolized contaminant $i$ (milligrams per liter), and $T_{ci}$ is the transformation capacity for cometabolized contaminant $i$ (milligrams of i per milligram of cells). Combining equations 3 and 4 results in the following:

$$dt = \frac{-(K_{si} + S_i)}{k_i\left[X_o - \frac{1}{T_{ci}}(S_{oi} - S_i)\right]S_i}\, dS_i \tag{5}$$

Equation 5 can be integrated over time and contaminant concentration for a batch reactor to yield

$$t = \frac{1}{k_i}\left(\left(\frac{K_{si}}{S_o/T_{ci} - X_o}\right)\ln\left\{\frac{S_i X_o}{\left[X_o - \frac{1}{T_{ci}}(S_{oi} - S_i)\right]S_{oi}}\right\} + T_{ci}\ln\left\{\frac{X_o}{\left[X_o - \frac{1}{T_{ci}}(S_{oi} - S_i)\right]}\right\}\right) \tag{6}$$

Since MMO catalyzes the oxidation of both TCE and CF, competitive inhibition would be expected in the presence of both compounds. This inhibition can be described as follows (5):

$$-dS_i/dt = \frac{k_i X S_i}{K_{si}\left(1 + \frac{S_j}{K_{sj}}\right) + S_i} \tag{7}$$

where $S_j$ is the concentration of competitive cometabolized compound $j$ at time $t$ (milligrams per liter) and $K_{sj}$ is the half velocity constant for $j$ (milligrams per liter).

It would also be reasonable to postulate that the overall activity of the cells would be diminished in proportion to the amount of each contaminant transformed but weighted by the respective transformation capacity for each compound. For two competitively cometabolized compounds, the following would result:

$$X = X_o - \frac{1}{T_{ci}}(S_{oi} - S_i) - \frac{1}{T_{cj}}(S_{oj} - S_j) \tag{8}$$

where subscripts $i$ and $j$ refer to the two different competitively cometabolized compounds. The rate of disappearance of each compound in a batch reactor with resting cells can then be calculated by substituting the term described above for cellular activity (equation 8) into the competitive-inhibition expression (equation 7) for each compound, yielding the following:

$$-dS_i/dt = \frac{k_i\left[X_o - \frac{1}{T_{ci}}(S_{oi} - S_i) - \frac{1}{T_{cj}}(S_{oj} - S_j)\right]S_i}{K_{si}\left(1 + \frac{S_j}{K_{sj}}\right) + S_i} \tag{9}$$

$$-dS_j/dt = \frac{k_j\left[X_o - \frac{1}{T_{ci}}(S_{oi} - S_i) - \frac{1}{T_{cj}}(S_{oj} - S_j)\right]S_j}{K_{sj}\left(1 + \frac{S_i}{K_{si}}\right) + S_j} \tag{10}$$

These equations can be solved simultaneously by using time-step analysis.

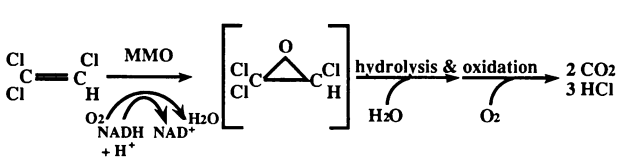


FIG. 2. Equation 2. Formation of epoxide by TCE oxidation and conversion of epoxide to mineralized end products.

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## MATERIALS AND METHODS

**Mixed culture development.** The development and maintenance of the mixed methanotrophic culture used in this study are described elsewhere (1). The culture was maintained in a 10-liter, continuously fed chemostat (growth reactor) with methane as the sole source of carbon, a measured average cell density of 2,500 mg/liter, and a net growth yield of 0.33 to 0.37 mg of cells per mg of $CH_4$ consumed.

**Experimental procedures. (i) Solutions.** Stock aqueous solutions of both CF and TCE were maintained in 160-ml glass bottles with Teflon-lined rubber septa and aluminum crimp tops. A 120-ml aliquot of Milli-Q water was added to approximately 10 ml of CF (99.5% pure Photrex Baker Reagent; J. T. Baker Chemical Co. Phillipsburg, N.J.) or TCE (99+% pure ACS reagent; Aldrich Chemicals Co., Milwaukee, Wis.) with glass beads added to promote mixing. A syringe was used to remove saturated aqueous solution, and care was taken to exclude non-aqueous-phase CF or TCE.

**(ii) CF and TCE transformation and methane consumption.** Transformation studies were conducted as previously described (1) in a 21°C environmental chamber with 62-ml bottles, mininert valves, 20 ml of medium or resting cells, three glass beads, and mixing at 200 to 300 rpm. Resting cells were used within 1 h of removal from the growth reactor, and all transformation studies were carried out in the absence of methane. Cell-free medium controls were used to track potential inadvertent compound disappearance. Formate-fed samples had 1 ml of cell solution replaced by 1 ml of 400 mM sodium formate solution immediately prior to solvent addition, resulting in a 20 mM formate final solution. Aqueous CF or TCE solution was added through the mininert valve with a 500-μl syringe to begin the experiment. CF and TCE transformations were measured as the change in total mass of compound in the bottle, using dimensionless Henry's law constants (at 21°C) of 0.11 and 0.31 for CF and TCE, respectively (23), to determine partitioning between the gaseous and liquid phases.

Methane consumption as a measure of cell activity was determined by a method similar to that used in the transformation studies, with bottles fitted with two septa (Teflon-lined silicone and Teflon-lined rubber septa) at 23°C and with mixing at 400 rpm. CF, TCE, and any volatile products were purged from bottles in which solvent transformation had been conducted previously by forced introduction of at least 10 volumes of air into the headspace. The cap was removed prior to resealing and methane introduction. Methane (3.0 ml) was injected into the bottle headspace, and the decrease in methane concentration with time was measured.

**(iii) Formate activity studies.** Formate studies were conducted similarly to the methane consumption studies, with 1.0 ml of 40 mM formate added to the bottles following treatments and headspace purging, yielding a 2 mM formate solution. The mixed-cell suspension (2.00 ml) was removed through the septa and added to 100 μl of 1 N sulfuric acid to halt the reaction. A 1.00-ml sample was then diluted to 50.0 ml with deionized water for formate measurement. Formate concentrations in control bottles containing equivalent amounts of cell-free medium were measured concurrently.

**Analytical procedures. (i) Culture density.** Culture density was determined by measuring the mass difference between preweighed aluminum foil dishes with 10 ml of liquid medium added and those with 10 ml of cell culture added after both sets were incubated overnight at 105°C.

**(ii) CF and TCE analyses.** CF and TCE concentrations were measured by headspace analysis as described previously (1) with the exception that 20-μl samples were withdrawn with a Hamilton automatic 0- to 20-μl adjustable syringe (Hamilton Co., Reno, Nev.). A Tracor MT-220 gas chromatograph equipped with a linearized electron capture detector and packed column (10% squalene on Chromosorb A/AW), maintained isothermally at 70°C with argon-methane (95%:5%) carrier gas, was used for analysis. An IBM-PC computer equipped with Nelson Analytical 3000 Series Chromatographic Data System software (Perkin-Elmer Nelson Systems Inc., Cupertino, Calif.) was used for peak integration.

Primary calibration standards in methanol were prepared with CF and TCE as described previously (1). Separate standards were maintained for CF and TCE, and calibration curves were produced daily at three or more concentration levels.

**(iii) Formate analysis.** Formate analysis was performed on a Dionex Series 4000i Gradient Chromatography system (Dionex Corp., Sunnyvale, Calif.) equipped with an Ionpac AS4A separator column and a conductivity detector using 5 mM sodium borate buffer eluent and Nelson software for peak integration. A 5-ml sample was injected through a 0.2-μm-pore-size Nylon 66 filter (Alltech, Inc., Deerfield, Ill.) with a glass multifit syringe. Quantitation was achieved by comparison with a calibration curve determined from six concentration levels of sodium formate solution prepared immediately prior to the analysis.

**(iv) Gas analysis.** Proportions of methane, oxygen, nitrogen, and carbon dioxide in a 200-μl headspace sample obtained with a Pressure-Lok gas-tight syringe were measured with a Fisher model 25V gas partitioner equipped with a thermoconductivity detector using helium carrier gas and calibrated with certified gas standards.

## RESULTS

In order to confirm the involvement of MMO in the CF transformation and to assess the extent of CF loss due to sorption onto cell walls, approximately 420 μg of CF was added to three sets of bottles: medium controls containing 20 ml of culture medium (no cells), acetylene controls containing 37 mg of resting cells in 20 ml of medium with a 25% acetylene atmosphere (99.6% purity, atomic absorption grade; Liquid Carbonics) to inhibit MMO activity (42), and active cells containing 37 mg of resting cells in 20 ml of medium. While the CF in the bottle of active cells decreased 60% within 4 h, the CF in the medium control was unchanged and that in the acetylene control decreased by only 10%. As with TCE transformation by this mixed culture (1), acetylene inhibition of CF transformation implies an MMO-catalyzed oxidation. Additionally, no adaptation time was required for the onset of CF transformation, even though the organisms had not been previously exposed to CF.

CF transformation by resting cells alone was initially fast but slowed to zero after 2 h (Fig. 3). $T_c$ of the resting cells alone was 0.0065 mg of CF per mg of cells, but with 20 mM sodium formate, $T_c$ increased to 0.015 mg of CF per mg of cells (measured by readdition of CF). Transformation did not cease because of diminished energy supply alone, since the second addition of sodium formate had little regenerating effect. This implies that like TCE, CF transformation has a toxic effect on methanotrophic resting cells. This was further confirmed by diminished methane consumption rates following CF transformation (Table 1).

A non-linear-regression analysis of the experimental data

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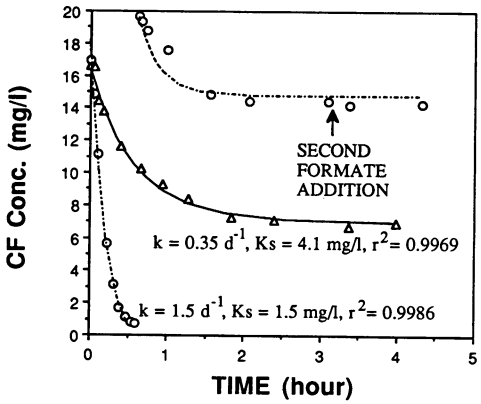


FIG. 3. Experimental data for CF transformation by methanotrophic resting cells (△) and formate-fed cells (○) shown with best-fit lines from the cometabolic transformation model and resultant rate coefficients using measured transformation capacities of 0.0065 and 0.015 mg of CF per mg of cells for resting cells and formate-fed cells, respectively. Ineffectiveness of second equivalent formate addition following cell inactivation is also shown.

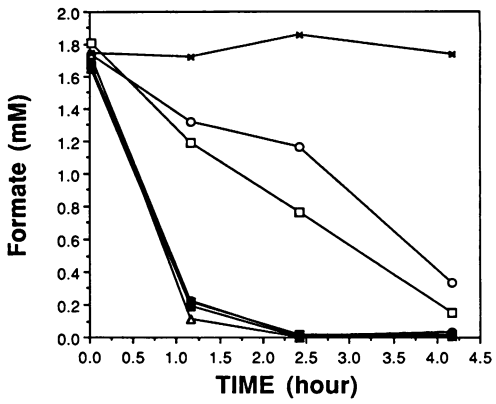


FIG. 4. Formate removal by methanotrophic resting cells after 3 h of exposure to CF (○ and ●) or TCE (□ and ■) (contaminant-exposed cells) or 3 h of incubation (positive controls) (△ and ▲). Closed symbols indicate cells which had been treated with acetylene prior to contaminant exposure in order to arrest MMO activity. ×, Medium control.

using measured transformation capacities with the cometabolic transformation model (equation 6) provided the model coefficients in Fig. 3, which are shown together with a comparison between the data and the model fit.

Determining whether the toxic effects observed during CF and TCE transformations were due to the compounds themselves or were associated with CF and TCE transformation products is not straightforward, since CF and TCE transformations began immediately, decreasing the CF and TCE concentrations and exposing cells to the transformation products as well. However, acetylene arrests MMO activity and thus prevents the transformation without adversely affecting other cell functions. Therefore, acetylene-treated cells can be used to observe the toxic effects of CF and TCE by themselves. However, use of acetylene for this purpose would preclude the use of methane oxidation as a measure of cell metabolic activity. Instead, formate oxidation can be an effective measure of cell activity since the reaction (i) is catalyzed by formate dehydrogenase (37), an enzyme unaffected by acetylene (13); (ii) is the final step in methane oxidation and therefore essential for normal cell metabolism; and (iii) is useful for methanotrophs to generate energy but not for cell growth.

To this end, 35 mg of resting methanotrophic cells in 20 ml of medium was added to a series of bottles. Half were acetylene treated by shaking the bottles for 20 min with a headspace of 10% acetylene. The acetylene was then purged from these bottles. Acetylene-treated cells and active cells were then exposed for 3 h to either 60 mg of CF per liter or 36 mg of TCE per liter. As expected, no significant transformation occurred with the acetylene-treated cells. However, 24% of the CF and 99% of the TCE were transformed by the active cells. The headspace of each bottle was then purged with air to remove all CF, TCE, or $CO_2$ present and to replenish the oxygen supply. The metabolic activities of the remaining cells were then determined by adding formate and measuring its disappearance over time. A medium control which was devoid of cells and positive controls containing either acetylene-treated cells or active cells which were not exposed to the contaminants were used for baseline measurements. Cells which had transformed either CF or TCE showed a significantly diminished formate consumption rate (Fig. 4) compared with those of both the acetylene-treated cells and the positive controls. Additionally, there was no significant difference in formate activity for the acetylene-treated and active positive controls, suggesting that acetylene treatment alone did not result in diminished formate activity. These results confirm that it is the transformation products of both CF and TCE rather than the CF and TCE themselves which are primarily responsible for the observed toxicity to this methanotrophic culture.

TABLE 1. Specific rate of methane consumption for cells after 4 h with or without exposure to 17 mg of CF per liter

| Kind of cells | Formate addition (mM) | Amt of CF transformed (μg) | Methane consumption rate (mg of $CH_4$/mg of cells/day) |
|---|---|---|---|
| Without CF | 0 | 0 | 0.300 |
| | 20 | 0 | 0.330 |
| CF exposed | 0 | 240 | 0.006 |
| | 20 | 520 | 0.024 |
| CF exposed, acetylene treated | 20 | 40 | 0.018 |

An additional experiment was conducted to evaluate the hypothesis that concurrent CF and TCE transformation can be described by competitive-inhibition kinetics. First, measurements of the disappearance of CF alone in the presence of 48.4 mg of resting cells in 20 ml of medium resulted in a $T_c$ of 0.0083 mg of CF per mg of cells, while a similar evaluation with TCE yielded a $T_c$ of 0.042 mg of TCE per mg of cells. Non-linear-regression analysis of these data with the cometabolic transformation model yielded $k$ and $K_s$ values for the model which are shown along with the respective experimental data for the individual compound transformations in Fig. 5. The disappearance of CF and TCE added simultaneously to resting cells was also measured. The parameters from the individual disappearance studies were entered into the cometabolic competitive-inhibition equations 9 and 10. The resultant simultaneous CF and TCE disappearance predictions are shown along with the experimental data in

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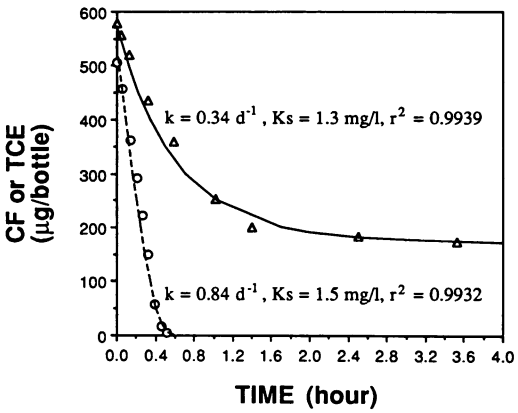


FIG. 5. Individual CF (△) and TCE (○) disappearance with resting methanotrophic cells along with cometabolic model lines and estimated coefficients using measured transformation capacities of 0.0083 mg of CF and 0.042 mg of TCE per mg of cells for CF and TCE, respectively.

Fig. 6. The agreement between model predictions and experimental observations is reasonable and supportive of the assumptions within the model. The presence of CF had a much greater impact on the TCE transformation rate and extent than the reverse, as was suggested by the model.

## DISCUSSION

Results for CF transformation by a resting methanotrophic mixed culture were similar to previous results for TCE transformation (1, 2) in that sharply declining transformation rates with time and the presence of a finite $T_c$ are observed. Thus, the same question of whether the rate declines are due to depletion of stored energy reserves or to toxic effects of CF or its transformation products is raised.

For the methanotrophic resting cells studied here, the transformation rate and $T_c$ for CF (0.34 day$^{-1}$ and 0.0083 mg of CF per mg of cells) were significantly lower than for TCE (0.84 day$^{-1}$ and 0.042 mg of TCE per mg of cells). Formate addition resulted in a significantly increased rate and capacity for CF, as was found previously for TCE (1), implicating depleted energy reserves as one factor in transformation

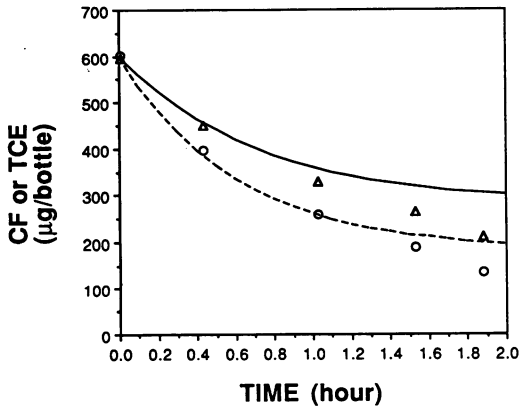


FIG. 6. Concurrent disappearance of CF (△) and TCE (○) with resting methanotrophic cells along with predictions computed by using the cometabolic competitive-inhibition model. ——, CF; — — —, TCE.

behavior. However, the failure of the addition of 20 mM formate to regenerate the exhausted CF oxidation activity of whole cells (Fig. 3) indicates that, similar to the case for TCE transformation (1), the finite transformation capacity is not solely a result of depleted energy reserves. Rather, the toxic effect of CF itself or its transformation products is also implicated. This is substantiated by the observed diminished methane consumption rate following CF transformation (Table 1) and is in agreement with the results of Janssen et al. (30), who reported that methanotrophic activity was inhibited by 8 mg of CF per liter. However, these results by themselves do not elucidate whether this toxicity is due to CF itself or is induced as a result of CF transformation.

The good fit between the cometabolic transformation model and the CF transformation data both with resting and formate-fed cells (Fig. 3) is further evidence that the implicit assumption of the model, i.e., that transformation decreases as a function of the amount of CF consumed, is appropriate. This implies that toxicity and electron donor supply play interacting roles in both CF and TCE transformation behavior, which is not surprising, since both reactions are MMO catalyzed, both require NADH and molecular oxygen, and both produce potentially toxic intermediates. The fit also reinforces the broader applicability of the cometabolic transformation model to a compound other than TCE. The fit of the model incorporating competitive inhibition to the concurrent CF and TCE disappearance data (Fig. 6) is further evidence that a single enzyme is involved in transformation of both compounds. The results suggest the potential applicability of the model to multiple contaminants.

An important finding from this study is that possible toxic effects of CF and TCE themselves, at concentrations of 60 and 36 mg/l, respectively, on the resting methanotrophic cells studied here, are insignificant compared with the toxic effects of resulting CF and TCE transformation products. This was clearly demonstrated through experiments in which acetylene was added to maintain high levels of CF and TCE and to prevent product formation so that CF and TCE toxicity alone could be determined. Cell activity was determined by the rate of formate oxidation. However, use of the formate oxidation rate for this purpose does have some limitations. Formate oxidation does not depend on MMO; consequently, measuring it would fail to detect changes in MMO-associated cell activity. However, studies conducted with purified MMO (19) suggest that MMO inactivation also results from TCE oxidation rather than from exposure to TCE itself.

Additionally, because of the occurrence of formate dehydrogenase in most methylotrophs (31, 37), those within the mixed culture used in this study which are MMO deficient might consume formate along with the methanotrophs and could be responsible to some degree for the residual formate-using activity of the TCE- and CF-exposed cells. Therefore, similar experiments conducted with pure methanotrophic cultures could lead to an even better resolution of this issue. Presently, it is possible to speculate that phosgene and TCE epoxide are responsible for the observed product toxicity of CF and TCE, respectively, since both compounds have been shown to exhibit irreversible binding to proteins thought to result in toxic behavior in mammalian systems (6, 39).

The results of this study have important implications for treatment process design. Analogous product toxicity could be expected for MMO oxidations of other halogenated compounds as well as transformations catalyzed by similar enzymes such as ammonia monooxygenase, which has recently been reported to catalyze oxidations of a range of

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chlorinated aliphatics including TCE and CF (3, 49); propane monooxygenase, which is also responsible for TCE oxidation (52); and toluene monooxygenase, which is present in TCE-oxidizing organisms (40). In order to design a treatment system for other chlorinated aliphatic compounds, transformation capacities need to be determined. In addition, this study has demonstrated that when these compounds occur as mixtures, interactions that affect both the transformation rate and capacity of the individual compounds are likely. The cometabolic competitive inhibition model presented here represents a first step in the quantification of these affects, but it needs more-thorough evaluation and extension to multiple-compound systems.

A possible treatment strategy to help optimize the competitive reactions involved is the dual-stage dispersed-growth reactor system proposed previously (2a) in which cells harvested from a growth reactor are transferred to a treatment reactor, where they are placed in contact with the waste stream and eventually discarded when their transformation activity approaches exhaustion. For fixed-film or in situ attached-growth treatment systems, product toxicity suggests a design in which cell growth outpaces the loss of activity due to toxicity. The addition of an exogenous energy source, such as formate, may be desirable to increase the overall transformation capacity of the cells if the regenerating growth rate is insufficient.

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