Adaptation of Aquatic Microbial Communities to Quaternary Ammonium Compounds

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Received 25 June 1985/Accepted 13 November 1985

The effects of long-chain (C12 to C18) quaternary ammonium compound (QACs) on the density, heterotrophic activity, and biodegradation capabilities of heterotrophic bacteria were examined in situ in a lake ecosystem. Monoalkyl and dialkyl substituted QACs were tested over a range of concentrations (0.001 to 10 mg/liter) in both acute (3 h) and chronic (21 day) exposures. In general, none of the QACs tested had significant adverse effects on bacterial densities in either acute or chronic studies. However, significant decreases in bacterial heterotrophic activity were noted in acute studies at QAC concentrations from 0.1 to 10 mg/liter. Chronic exposure of lake microbial communities to a specific monoalkyl QAC resulted in an adaptive response and recovery of heterotrophic activity. No-observable-effect level in the adapted populations was >10 mg/liter. Chronic exposure also resulted in significant increases in the number and activity of bacteria capable of biodegrading the material. The increase in biodegradation capability was observed at low (microgram per liter) concentrations which are approximately the same as realistic environmental levels. In general, our studies indicated that exposure of lake microbial communities to QACs results in the development of adapted communities which are less sensitive to potential toxic effects and more active in the biodegradation of these materials.

Surface-active monoalkyl and dialkyl quaternary ammonium compounds (QACs) with alkyl chain lengths from C12 to C18 represent a commercially important class of chemicals in the United States. These materials have a variety of industrial and consumer applications including use in drilling muds, fabric softeners, hair conditioners, emulsifying agents, and sanitizers/disinfectants. Production volumes for QACs in the United States have increased steadily in recent years from approximately 100 million lbs. (45 x 106 kg) in 1979 to more than 150 million lbs. (68 x 106 kg) in 1984 (2). There is a relatively extensive data base concerning the effects of QACs on aquatic organisms (12, 14, 16, 24). There is also a comprehensive review which summarizes available data on the fate and toxicity of QACs in wastewater treatment systems. However, with the exception of a few studies (1, 20, 21, 28–30), relatively little information is available on the fate and microbial effects of QACs in natural water systems. Previous work on the fate of QACs has largely concerned with their removal in wastewater treatment systems (3, 6, 7, 11, 13, 25, 31, 37, 40), whereas most studies of microbial effects have concentrated on the disinfectant or germicidal activities of these materials on specific microbial species (5). In general, the potential impacts of QACs on receiving waters and their indigenous microbiota have not been extensively studied, even though they have antimicrobial properties and have the potential to reach such environments associated with wastewater effluents or industrial discharges.

In this work, we studied the fate of QACs in a natural water system and their effects on the metabolic activities of indigenous microbial communities. These studies were conducted in situ in a lake ecosystem with a series of detergent-related QACs.

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MATERIALS AND METHODS

Test materials. Characterization and purity data for the QACs which were used are summarized in Table 1. Unlabeled QACs were used in studies of microbial effects to dose test chambers, whereas a specific 14C-labeled QAC (dodecyltrimethylammonium chloride [DTMAC]) was used in biodegradability/fate studies. [14C]DTMAC (2.36 MBq/mg) was obtained from Amersham Corp., Arlington Heights, Ill. Radiolabeled [U-14C]glucose (59.2 MBq/mg; Amersham) was used to measure bacterial heterotrophic activity in microbial effects studies.

Study area. In situ lake studies were conducted in Acton Lake, located in Preble and Butler Counties, Ohio, during the summer of 1983 (Fig. 1). Acton Lake is a 250-ha reservoir located approximately 60 km northwest of Cincinnati. The lake receives no direct wastewater discharge but is exposed to potential contaminants via septic tank seepage and agricultural runoff. The limnology and phytoplankton of Acton Lake were previously described (23).

Bacterial heterotrophic activity. The effects of a series of QACs on the density (numbers) and heterotrophic activity of bacterial communities in Acton Lake was determined in short-term acute (3 h) studies. The effects of a specific QAC, DTMAC, on these parameters were measured in both short-term acute and long-term chronic (21 day) studies. Total microbial numbers were determined by the acridine orange direct-count (AODC) procedure (10) as modified by Kirchman and Mitchell (15) to count both free and particle-bound cells. Bacterial heterotrophic activity was determined by the turnover time-tracer approach (8) in 100-ml serum bottles containing 10-ml samples of lake water with 0.01 μg of [14C]glucose per liter as the test substrate. The glucose turnover time (Tl), i.e., the time required for complete metabolism of glucose ([14C] uptake + [14CO2] at its in situ concentration, was calculated by the equation Tl = tf, where
TABLE 1. Quaternary ammonium compounds used in fate and effects studies

<table>
<thead>
<tr>
<th>Compound</th>
<th>Purity (%)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTMAC</td>
<td>98</td>
<td>CH₃⁻N⁺⁻CH₃₂(CH₂)₉CH₃Cl⁻</td>
</tr>
<tr>
<td>TTMAC</td>
<td>95</td>
<td>CH₃⁻N⁺⁻CH₃₂(CH₂)₁₄₂CH₃Cl⁻</td>
</tr>
<tr>
<td>DTDMAC</td>
<td>84</td>
<td>CH₃⁻N⁺⁻CH₃₂(CH₂)₁₄₂CH₃Cl⁻</td>
</tr>
<tr>
<td>[¹⁴C]DTMAC</td>
<td>98</td>
<td>CH₃⁻N⁺⁻¹⁴CH₃₂(CH₂)₉CH₃Cl⁻</td>
</tr>
</tbody>
</table>

$t$ is the incubation time (hours) and $f$ is $(¹⁴C$ uptake $+ ¹⁴CO₂)/¹⁴C$ added.

Short-term acute studies were conducted by collecting lake water from the photic zone (depth of 1 to 2 m) and distributing it to triplicate 300-ml biochemical oxygen demand bottles containing various concentrations of QACs (0.001 to 10 μg/liter). Triplicate controls contained water but no QACs. The bottles were incubated in the lake at a depth of approximately 1 m for 3 h and were then removed and assayed by the AODC procedure and for glucose $T_r$. Water samples for AODC determinations were preserved with 10% Formalin buffered with 100 mM sodium phosphate (pH 7.0). Significant differences in AODCs and glucose turnover times between control and test bottles were determined at the 95% confidence level by analysis of variance. Concentrations of QACs in water samples preserved with 1% Formalin were measured by the method of Wee and Kennedy (39). The maximum variation between nominal (added) and measured concentrations was approximately 30%, with typical variations of ±10%.

Long-term chronic studies were conducted for a specific QAC, DTMAC, also by using water collected from the photic zone. Lake water was pumped into large polyethylene tubs and thoroughly mixed by mechanical stirrers. The water was then distributed to duplicate sterile 2-liter polycarbonate carboys containing various concentrations of DTMAC; a paired control carboy containing no test material was included with each set of duplicate test carboys. The test material was mixed with the water, the carboys were capped, and each set of three carboys was suspended in the lake (at a 1-m depth) for a period of 21 days. At the end of the incubation period, water samples were collected from control and test carboys and assayed by the AODC procedure and for glucose $T_r$ relative to unexposed controls. Significant differences in AODCs and glucose turnover times between control and test carboys were determined at the 95% confidence level by analysis of variance. Concentrations of DTMAC were measured at intervals during the test period as previously described (39).

Biodegradability studies. The kinetics of biodegradation of $[¹⁴C]DTMAC$ in Acton Lake water were measured by using both biodegradation potential and time course techniques. Heterotrophic biodegradation kinetic studies were conducted as described by Parsons and Strickland (26), as modified by Wright and Hobbie (42), Hobbie and Crawford (9), and Pfaender and Bartholomew (27). Briefly, various concentrations of DTMAC (approximately 2 to 1,000 μg/liter) were incubated at 20°C in 10 ml of lake water for short periods of time, and the fraction of radiolabel assimilated plus that metabolized to $¹⁴CO₂ (¹⁴C$ uptake $+ ¹⁴CO₂)$ was determined. Data for the rates of degradation (nanograms per liter per hour) at various added substrate concentrations were analyzed by the following equation, which is hyperbolic saturation curve of the Michaelis-Menten type:

$$\nu = \frac{f \cdot A}{t} = \frac{V_{max} \cdot A}{K_a + A}$$

where $f$ is $(¹⁴C$ uptake $+ ¹⁴CO₂)/¹⁴C$ added, $t$ is the incubation time (hours), $A$ is the added substrate concentration (micrograms per liter), $V_{max}$ is the maximum degradation rate (nanograms per liter per hour), and $K_a$ is the half-saturation constant (micrograms per liter) where $ν = 0.5 \cdot V_{max}$. Parameter estimates for $V_{max}$ and $K_a$ were obtained by doing a least squares analysis with a nonlinear computer program as previously described (17). Specific activity indices (SAIs) were calculated by dividing $V_{max}$ by the AODC (41) or by the most probable number (MPN) of degraders.

Time course studies were conducted as previously described (19) by using replicate 2-liter Erlenmeyer flasks each containing 1 liter of lake water and $[¹⁴C]DTMAC$ at an initial concentration of 100 μg/liter. The flasks were incubated at 23 ± 2°C in a constant-temperature room and continually agitated (150 rpm) on a rotary platform shaker. At various intervals, subsamples (10 ml) were taken from the solutions and filtered through a 0.2-μm polycarbonate filter to remove particulate matter. Each filter was washed with 5 ml of 50% ethanol, and the 15-ml filtrate was added to a 125-ml biometer flask. The filtrate was acidified with 1 ml of concentrated HCl to remove $¹⁴CO₂$, which was trapped in 2 ml of 1.5 N KOH in the biometer side arm. Subsamples from
the base trap (1 ml) and filtrate (10 ml) were then analyzed by liquid scintillation spectrometry to quantitate the amount of radioactivity as $^{14}$CO$_2$ and as $^{14}$C-activity in solution, respectively. Data for the amount of cumulative $^{14}$CO$_2$ produced or $^{14}$C activity removed from solution (as a percentage of $^{14}$C added) were analyzed by nonlinear regression models to estimate the first-order rate constants ($k_f$) for mineralization and removal, normalized for the extent of $^{14}$CO$_2$ production or $^{14}$C removal observed. Parameter estimates for $k_f$ were generated by iterative techniques by using a least squares analysis and a nonlinear computer program as previously described (17).

**Enumeration of DTMAC degraders.** A modification of the method of Lehmicke et al. (22) was used to determine the MPN of bacteria capable of metabolizing $^{14}$C]DTMAC. Filter-sterilized lake water supplemented with the following constituents served as the growth medium: NH$_4$NO$_3$ (0.1%), K$_2$HPO$_4$ (0.08%), Na$_2$HPO$_4$ (0.1%), NaCl (0.1%), CaCl$_2$·2 H$_2$O (0.002%), NaHCO$_3$ (0.05%). $^{14}$C]DTMAC (20 µg/liter) was added to each of five replicate serum bottles containing serial (1:10) dilutions of lake water. The bottles were incubated quiescently in the dark for 5 to 6 weeks at 20°C. Individual bottles were acidified, and the $^{14}$CO$_2$ produced was collected on fluted wicks soaked with 1.5 N KOH.

![FIG. 1. Acton Lake study site.](http://aem.asm.org/)

### TABLE 2. Effects of acute and chronic exposure to QACs on the activity of heterotrophic bacteria in Acton Lake

<table>
<thead>
<tr>
<th>Nominal QAC concn (mg/liter)</th>
<th>Glucose turnover time (h)$^a$</th>
<th>Acute exposure to:</th>
<th>Chronic exposure to DTMAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DTMAC</td>
<td>TTMAC</td>
</tr>
<tr>
<td>0 (control)</td>
<td>16 (A)</td>
<td>36 (A)</td>
<td>10 (A)</td>
</tr>
<tr>
<td>0.001</td>
<td>17 (A)</td>
<td>22 (A)</td>
<td>ND</td>
</tr>
<tr>
<td>0.01</td>
<td>12 (A)</td>
<td>38 (A)</td>
<td>15 (A)</td>
</tr>
<tr>
<td>0.1</td>
<td>70 (B)</td>
<td>320 (B)</td>
<td>12 (A)</td>
</tr>
<tr>
<td>0.5</td>
<td>1,305 (C)</td>
<td>ND</td>
<td>17 (A)</td>
</tr>
<tr>
<td>1.0</td>
<td>1,854 (C)</td>
<td>867 (B)</td>
<td>349 (B)</td>
</tr>
<tr>
<td>5.0</td>
<td>4,781 (D)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10.0</td>
<td>ND</td>
<td>1,301 (C)</td>
<td>2,513 (C)</td>
</tr>
</tbody>
</table>

$^a$ Different letters in parentheses indicate significant differences between figures in a given column ($P < 0.05, n = 2$ to $4$).

$^b$ ND. Not done.

Replicates which evolved an amount at least twice that of the killed control were counted as positive. The MPNs of degraders and the associated 95% confidence intervals were calculated by a nonlinear microcomputer program (4).

### RESULTS

**Microbial effects studies.** (i) **Short-term acute studies.** The results of dose-response studies to determine the effects of monoalkyl and dialkyl QACs on the metabolic activity of heterotrophic bacteria in short-term acute studies are summarized in Table 2. The various QACs were tested several times over a 4-month period (June to September 1983), and the data shown are means for all experiments.

Bacterial densities (measured by AODC) were relatively constant across tests (mean ± 30%) and exhibited no consistent, significant change as a function of QAC dose (data not shown). Damage to bacterial cell membranes resulting in lysis, therefore, did not occur for any QAC tested at any dose level. However, significant dose-dependent decreases in bacterial heterotrophic activity (i.e., increased glucose $T_I$) were observed in short-term tests at QAC concentrations from 0.1 to 10 mg/liter. The first-effect concentration (FEC) for inhibition of heterotrophic activity was the same for the monoalkyl QACs DTMAC and tallowtrimethylammonium chloride (TTMAC) (approximately 0.1 mg/liter). The FEC for the dialkyl QAC dialkowdimethylammonium chloride (DTDMAC) was about 10-fold higher (approximately 1.0 mg/liter) indicating that it was significantly less toxic to microbial communities. For all three QACs, however, significant reductions (5- to 100-fold) in glucose metabolism occurred at the FEC and higher concentrations. At toxic-effect levels, therefore, ecologically significant responses were observed.

(ii) **Long-term chronic studies.** The results of dose-response studies to determine the effects of a specific QAC, DTMAC, on glucose turnover in long-term chronic studies are summarized in Table 2. The chronic studies were conducted to determine if acute effects on heterotrophic activity would be attenuated and if recovery and adaptation of microbial communities to a specific QAC could occur. The monoalkyl QAC DTMAC was chosen for the chronic studies because it consistently had the lowest effect levels in the acute studies.

Bacterial densities in the long-term studies, as in the short-term studies, were unaffected by a wide range of DTMAC concentrations (0.001 to 10 mg/liter). In contrast to the acute studies, however, no significant adverse effects on
bacterial heterotrophic activity were observed in the chronic studies at DTMAC dose levels as high as 10 mg/liter. The highest DTMAC dose tested in chronic studies was approximately 100-fold greater than its FEC in acute studies. However, rates of glucose metabolism in all test units were comparable to or greater than those in the control units. These results indicate that chronic exposure of microbial communities to DTMAC leads to the selection of more tolerant bacterial species and to overall recovery of heterotrophic activity in the microbial community.

**Biodegradability studies.** (i) **Time course studies.** The kinetics of biodegradation of DTMAC in Acton Lake water before and after a period of exposure are shown in Fig. 2. Biodegradation of DTMAC (100 μg/liter) upon initial exposure exhibited about a 24-h lag phase before of measurable production of 14CO2 or removal of 14C activity from solution occurred. After this lag phase, both processes followed apparent first-order kinetics. The estimated half-lives (t1/2) for mineralization and removal based on the observed first-order rate constants were 150 and 91 h, respectively. The half-life for removal was somewhat shorter than that for mineralization because of incorporation of some radiolabel into biomass. When additional DTMAC (100 μg/liter) was respiked into the same lake water (day 15), degradation occurred immediately with no lag phase. The rate of DTMAC removal after the respike (t1/2 = 20 h) was significantly faster (four- to fivefold) than the initial removal rate (t1/2 = 90 h). Prior exposure to DTMAC, therefore, resulted in an adaptive response in the microbial community and enhanced biodegradation of DTMAC. The mechanism responsible for this was further studied by using short-term biodegradation assays as indicated below.

(ii) **Heterotrophic activity studies.** The results of studies to characterize the mechanism for the development of biodegradation capability in Acton Lake water are summarized in Table 3. Lake water was exposed to various concentrations of unlabeled DTMAC for 21 days, and the maximum biodegradation rates (Vmax) were then determined by using labeled DTMAC at the various exposure concentrations. The numbers of total (measured by AODC) and 14C/DTMAC-degrading (measured as MPN) bacteria were also determined to allow calculation of Vmax SAI. The SAIs were used to compare 14C/DTMAC biodegradation rates at the different exposure concentrations on a per-cell basis. Chronic exposure of bacterial populations to DTMAC resulted in a significant adaptive response relative to the biodegradation of this material (Table 3). Maximum biodegradation rates (Vmax) increased as the DTMAC dose level increased and were significantly higher (2 to 5 orders of magnitude) than those for the unexposed controls. The increase in biodegradation rates at low dose levels (0.001 and 0.1 mg/liter) was primarily due to the increased numbers of bacterial cells active on 14C/DTMAC. This is reflected in the SAIs, which remained relatively constant at both dose levels. The increased degradation rate at the high dose level (10 mg/liter) reflected a significant increase in both numbers and per-cell activity since SAIs were higher. For all DTMAC dose levels, a significant increase in the MPN of DTMAC-degrading bacteria occurred relative to unexposed controls.

### DISCUSSION

Adaptation of aquatic microbial communities to xenobiotic chemicals can be separated into fate and effect components. Of the two components, the fate adaptation component has received more attention (35, 36) and refers to a process whereby the rate of biodegradation of a chemical is significantly increased as a result of prior exposure to that.

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**TABLE 3. Development of DTMAC biodegradation capability in Acton Lake water as a function of DTMAC exposure**

<table>
<thead>
<tr>
<th>Nominal DTMAC exposure concn (mg/liter)</th>
<th>Vmax (ng/liter per h)</th>
<th>MPN of DTMAC degraders (cells/ml [10^9])</th>
<th>AODC (cells/ml [10^9])</th>
<th>SAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control, day 0)</td>
<td>&lt;0.01</td>
<td>0.5 (0.2–1.5)</td>
<td>5.1 (3.1–7.2)</td>
<td>ND</td>
</tr>
<tr>
<td>0 (control, day 21)</td>
<td>&lt;0.01</td>
<td>0.1 (0.05–0.4)</td>
<td>5.7 (2.4–9.1)</td>
<td>ND</td>
</tr>
<tr>
<td>0.001</td>
<td>505 (257–752)</td>
<td>4.9 (1.6–15.3)</td>
<td>2.6 (0.9–4.3)</td>
<td>1.03</td>
</tr>
<tr>
<td>0.1</td>
<td>722 (380–1,065)</td>
<td>4.9 (1.6–15.3)</td>
<td>3.3 (1.1–5.5)</td>
<td>1.473</td>
</tr>
<tr>
<td>10.0</td>
<td>&gt;10,000¹</td>
<td>21.6 (8.1–57.7)</td>
<td>7.0 (3.1–10.9)</td>
<td>&gt;14.3</td>
</tr>
</tbody>
</table>

¹ Values in parentheses are 95% confidence intervals of parameter estimate.
² Values in parentheses are 95% confidence intervals of mean value.
³ Detection limit of technique.
⁴ ND, Not done.
⁵ Saturation kinetics not observed at highest DTMAC concentration tested (1.022 μg/liter).
chemical. Fate adaptation processes are important since they facilitate the rapid removal of chemicals from the environment and minimize the potential for exposure of sensitive aquatic biota to the chemicals. However, for materials which have distinct antimicrobial properties, a second adaptation process which results in the selection of more tolerant microbial populations may also be important. This process tends to minimize potential adverse effects on primary and secondary productivity within an ecosystem.

Based on the results of our studies, both types of adaptation processes appear to be important in mitigating potential adverse environmental responses to QACs. We used the turnover time-tracer approach to measure dose-dependent decreases in glucose metabolism caused by DTMAC. This approach is valid, even across different water samples, if the background concentration of glucose is in the linear or first-order region of the glucose velocity curve and the concentration of added 14C tracer is only a small fraction (1% or less) of the total glucose pool present. In short-term acute studies, the monoalkyl QACs DTMAC and TTMAC exhibited relatively low FEs (0.1 mg/liter). However, chronic exposure of microbial communities to DTMAC resulted in essentially complete recovery of microheterotrophy and no discernible toxic effects at concentrations 2 orders of magnitude higher than its acute FE. To some extent, this recovery may have been a result of biodegradation of DTMAC during the 21-day test period; this was largely unavoidable in the batch test system which was used (Fig. 2). Analysis for DTMAC after 10 days of incubation showed that carboys dosed with 0.5 mg/liter or greater still had concentrations at or above the EFC for the acute test (data not shown). Measured DTMAC concentrations at the end of testing were about 30% of nominal (3.0 mg/liter) at the high dose level (10 mg/liter) and below detection limits at the remaining dose levels. This means that actual exposure concentrations in chronic studies were less than the nominal concentration and that the effective exposure period was greater than 10 but less than 21 days. However, both the concentration and the length of the exposure period were sufficient to elicit a significant adaptive response which was consistent for all dose levels.

Chronic exposure of microbial communities to DTMAC in fate studies also resulted in a significant adaptive response with respect to the development of biodegradation capability. For all dose levels, substantial increases in the number and biodegradation activity of DTMAC-degrading bacteria occurred relative to unexposed controls. Maximum degradation rate and number of DTMAC degraders increased 1 to 5 orders of magnitude even at initial DTMAC concentrations as low as 1 µg/liter. The increase in the maximum degradation rate at the high dose level was a result of increases in both the number of degraders and the activity per cell (Table 3). Acclimation at the low concentrations was a result of the increased number of DTMAC degraders in the community. The increase in the MPN at the lowest dose level was probably not due to growth on the DTMAC alone. This concentration is below the threshold concentrations for growth of other bacteria (33, 38). It may be that these cells were induced or derepressed for activity by the low level of DTMAC and grew on the other dissolved organic matter in the lake water. Organic matter, even at ppb (nanogram per milliliter) concentrations, stimulates both bacterial growth and biodegradation of organic chemicals (32, 34, 38). Further study is required to elucidate the mechanism(s) involved in the adaptation of communities exposed to low concentrations of chemicals.

Adaptation of microbial communities to DTMAC at low, environmentally relevant concentrations has significant implications for this material and for related QACs. It indicates that biodegradation is an effective removal mechanism in dilute natural systems where chemical concentrations are often in the microgram per liter range. We observed adaptation of microbial populations to DTMAC at nominal concentrations as low as 1 µg/liter. This concentration is well below the analytical detection limit for this material (5 µg/liter) and below previously reported values for other industrial chemicals. In practical terms, therefore, a threshold response for DTMAC could not be demonstrated.

Lack of a significant threshold concentration was also observed by Larson and Davidson (18) for nitroloiacetic acid in natural waters. These results, as well as those reported above, are in contrast to previous reports by Spain and coworkers (35, 36) who found that a minimum concentration threshold existed for some industrial chemicals. These thresholds were 10 to 50 µg/liter in freshwater systems. From the limited number of studies, it appears that the existence of threshold concentrations for adaptation is dependent not only on the environment studied (35) but also on the structure of the chemical. More data are required to characterize the response of microbial communities to trace levels of xenobiotics and the factors which affect that response.

In assays of short-term effects, monoalkyl QACs (DTMAC and TTMAC) and the dialkyl QAC (DTDMAC) significantly inhibited bacterial heterotrophic activity at initial concentrations of 0.1 and 1.0 mg/liter, respectively. The relative order of toxicity was DTMAC < TTMAC < DTMAC. Significant adverse effects on bacterial density were not observed for any of the materials at dose levels as high as 10 µg/liter. Chronic exposure of lake microbial communities to DTMAC resulted in an adaptive response and recovery of heterotrophic activity. No significant adverse effects on bacterial density or activity were observed in chronic studies at the highest dose level tested (10 mg/liter). Chronic exposure of microbial communities to a range of DTMAC concentrations (0.001 to 10 mg/liter) also resulted in an adaptive response in fate studies, and significant increases in the number and activity of DTMAC-degrading bacteria were observed relative to levels for unexposed controls. The development of DTMAC-degrading capability occurred at concentrations in the microgram per liter range which are realistic environmental levels.

ACKNOWLEDGMENTS

We thank J. A. Staubach, B. S. Schwab, and Theresa Williams for their excellent technical assistance.

This work was funded in part by a grant to R.M.V. from the University of Dayton Research Council.

LITERATURE CITED