Methyl chloride (CH$_3$Cl) production in phytoplankton cultures

**Abstract**—Unialgal nonaxenic cultures of seven species of phytoplankton, including both warm- and cold-water organisms, were examined under halocarbon-clean conditions for the production of CH$_3$Cl. Incubations of all species showed CH$_3$Cl increases significantly greater than in the control medium. The magnitude of increase varied with the phytoplankton species present and in some cases also with growth stage. Longer time series showed CH$_3$Cl continued to increase after the death of all the phytoplankton cells. Although direct production as a by-product of phytoplankton metabolism cannot be ruled out earlier in the time series, indirect chemical and (or) biotic reaction of algal-associated organic matter is required to explain this continued increase. Characterization of the role of bacteria in both pre- and postsenescent stage CH$_3$Cl production will require comparable experiments carried out under axenic conditions.

Recognition of the role played by volatile organohalogen compounds in the regulation of stratospheric ozone (Molina and Rowland 1974) prompted extensive study into cycles of both anthropogenic and natural halomethanes in the environment. CH$_3$Cl was found to be the dominant organochlorine compound in the troposphere (Singh et al. 1977; Rasmussen et al. 1980). The lack of interhemispheric gradient in the free tropospheric mixing ratio of CH$_3$Cl (Rasmussen et al. 1980; Koppmann et al. 1993) suggested a widespread natural source. Both CH$_3$Cl and CH$_3$Br have lifetimes within the troposphere (1.7 yr—Koppmann et al. 1993; Mellouki et al. 1992) sufficient to allow some fraction of surface inputs to reach the stratosphere.

Measurements by Lovelock (1975) first indicated that
Glacialis air in regions typified by high bioactivity (Rasmussen et al. 1982). We report here the production of CHCl by concentrations of CHI have been observed in seawater and by release from coastal macroalgae. Above-average concentrations of two cold-water phytoplankton species—Porosira glacialis and Nitzschia sp.—were observed in the natural production of some volatile organohalogens by some species of macroalgae is well documented (e.g. Gschwend et al. 1985; Manley et al. 1992). Macraalgal production of polybrominated methanes is an important source of organobromine to the troposphere (Gschwend et al. 1985; Manley et al. 1992). However, as calculated by Manley and Dastoor (1987), release of monohalomethanes by coastal macroalgae is unlikely to be important on a global scale.

The first direct evidence of phytoplankton involvement in the natural production of some volatile organohalogens was CHBrCl emission observed in laboratory incubations of microalgal communities collected from beneath arctic annual ice (Sturges et al. 1992). Release of CHBrCl, CHBr2Cl, and CHBrCl2 has also been detected in laboratory incubations of ice-algae from the antarctic (Sturges et al. 1993) and in situ incubations in the arctic (Moore et al. 1994). The influence of bacteria and grazers present in these natural populations on halocarbon emission is not yet clear. Production of CHBr3, CHBr2Cl, CHBrCl2, and CH2Cl2 has been observed in unialgal laboratory cultures of two cold-water phytoplankton species—Porosira glacialis and Nitzschia sp. (CCMP 580) (Tokarczyk and Moore 1994). To our knowledge, only circumstantial evidence has been published supporting a phytoplankton role in the production of the monohalomethanes. The widespread supersaturation of CHCl has been observed in unialgal cultures of several species of phytoplankton—the first direct evidence supporting a phytoplankton role in oceanic CHCl production.

Nonaxenic cultures of several species of phytoplankton have been examined for the production of CHCl. About 450 ml of autoclaved f/2 or f/8 medium (Guillard 1975) in a glass vessel was purged with ultra-high purity (UHP) air/CO2 (~0.1% CO2) for 36 h to remove halocarbon contaminants. Following inoculation with 2–3 ml of algal culture, gentle purging was continued for a further 3 h, then the vessel was sealed. Warm-water species (diatoms: Odontella mobilensis, Phaeodactylum tricornutum, Thalassiosira weissflogii; prymnesiophyte: Isochrysis galbana) were incubated at 20°C under “cool-white” fluorescent tubes (~70 μmol quanta m−2 s−1) with a diel L/D cycle of 16:8. Cold-water phytoplankton (diatoms: Porosira glacialis, Nitzschia seriata, Nitzschia sp. CCMP 580) were held under constant illumination of 10 μmol quanta m−2 s−1 and incubated at 4°C. Samples of medium were withdrawn every 2–3 d through a metal needle into vacuum-baked (100°C, minimum 24 h) all-glass syringes for immediate determination of CHCl. During sample removal, a low flow of air/CO2 was maintained to prevent entrance of contamination from the atmosphere. The gas flow was continued for a further 3 min to supply CO2. Care was taken to minimize the numbers of phytoplankton removed from the vessel. In the case of the cold-water species and O. mobilensis, the cells settled at the bottom of the vessel, and negligible numbers were withdrawn during sampling. T. weissflogii, P. tricornutum, and I. galbana, however, were dispersed throughout the medium volume, and removal of cells could not be avoided. It is unknown whether the physical disruption of the cells of these three species during the purge step of the analysis results in additional release of CHCl. All species except N. seriata were originally obtained from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton. Analysis of CHCl was by purge-and-trap gas chromatography with oxygen-doped electron capture detection (ECD). The method used was modified from Tait et al. (1994) (Fig. 1). A 10-ml sample of culture medium was injected directly through a multiport valve into the purge vessel where it was purged for 5 min (40 ml min−1) with UHP helium onto a Porapak-Q trap held at 0°C (ice/water mixture). The trap was desorbed (100°C) into the gas chromatograph (HP 5890), where it was separated isothermally (70°C) on a 30 m GS-Q megabore column (J&W Scientific) with a helium carrier flow of 6 ml min−1. We doped the UHP N2 make-up gas with 0.5% oxygen (Grimstad and Miller 1978, 1981; Rasmussen et al. 1980) to increase the sensitivity of the ECD (275°C) to CHCl and extend the linear calibration range. The resulting detection limit was 7 pM, and precision was ±4% at the 200 pM level. The presence of CHCl in our phytoplankton cultures has been confirmed by GCMS.

Standards were prepared by injecting 20 μl of pure CHCl vapor (Aldrich Chem. Co.) through a Teflon-backed septum into 500 ml of prepurged (UHP N2, cooled (2–3°C) Super-Q water contained in a sealed glass vessel. A stir bar ensured rapid mixing and enhanced the rate of vapor dissolution. The low temperature and the high ratio of liquid to headspace volume within the vessel resulted in negligible partitioning of the injected CHCl into.
the gas phase. A time series of the standard that started 5 min after injection of the pure vapor into the vessel showed no significant change in peak area over a 12-h period, indicating that not only was equilibrium reached quickly within the vessel but also that the integrity of the standard was maintained over this time period. Precision of standards day to day was ±7% (C.V.).

After gentle agitation to disperse cells, samples were also withdrawn from the incubation vessels for light microscopy (Fuchs Rosenthal counting grid) and Coulter Counter cell counts to follow the growth of the cultures. Acridine orange epifluorescence microscopy (Hobbie et al. 1977) was used on several occasions to determine numbers of bacteria.

Unequivocal interpretation of field results is often hindered by the complex, dynamic nature of the ocean waters being sampled. Monospecific incubations of phytoplankton offer a more controlled environment in which to investigate possible ways phytoplankton may contribute to the CH₃Cl pool. The primary goal of these experiments was to determine whether any CH₃Cl release could be detected. Initially, high nutrient concentrations (f/2) were used to promote elevated cell densities. Later incubations with nutrient levels reduced to a quarter the former level allowed shorter experiment times without early nutrient limitation. The pH in all cultures at time zero was between 7.6 and 7.9. Media were not buffered, and the pH rose to 9.0 during the exponential and early stationary phases in the densest cultures.

Figure 2A shows the concentration of CH₃Cl measured in cultures of T. weissflogii and P. tricornutum. Production was slower during the exponential growth phase and increased with the onset of the stationary phase. Highest rates of CH₃Cl increase in the T. weissflogii culture were seen during and following phytoplankton senescence. No significant CH₃Cl production was observed in the control medium. Slow increases during the phytoplankton exponential growth phase compared with later in the time series were seen in repeat cultures of P. tricornutum and T. weissflogii and also in a culture of Nitzschia sp. (CCMP 580) (Fig. 2B). Unlike the T. weissflogii culture, however, production of CH₃Cl in the presence of CCMP 580 leveled off during the senescent phase. Incubations of N. seriata and P. glaciella that were run parallel to CCMP 580, although showing CH₃Cl levels significantly greater than the control, exhibited a lower, more uniform rate of CH₃Cl increase (Fig. 2B). The magnitude and pattern of CH₃Cl production depended on the species of phytoplankton present in the vessel.

Analysis of cultures of two of the warm-water species, O. mobiliensis (Fig. 3) and T. weissflogii (Fig. 2A), were continued after the death of all the phytoplankton cells. In both cases, CH₃Cl levels continued to increase. The extended incubation of O. mobiliensis still showed further CH₃Cl increase 2–3 weeks later. A dense culture (36,000 cells ml⁻¹) of O. mobiliensis was filtered (3-μm Nuclepore) under gentle vacuum to remove the phytoplankton cells (dimensions, ~50 × 75 μm) and leave only bacteria and algal exudates. The resulting filtrate was then purged of volatile halocarbons (air/CO₂) and incubated in a closed culture vial. The filtration step removed 80–90% of the bacteria, probably due to adhesion to phytoplankton cells. However, CH₃Cl showed a significant increase relative to a similarly filtered control medium (Fig. 4). Although
direct production of CH$_3$Cl by the phytoplankton cannot be ruled out in the full culture, indirect reactions (chemical or bacterially mediated) of algal organic compounds are required to account for these results.

Two indirect pathways of CH$_3$Cl production in seawater have been suggested. Zafiriou (1975) demonstrated that chloride substitution of CHJ was a mechanism of CH$_3$Cl formation in seawater. However, when the rate constants he measured in the laboratory are applied to the average level of CHJ throughout much of the open ocean (1–3 pM; Lovelock 1975; Singh et al. 1983), <5% of the CH$_3$Cl flux estimated to come from oceanic sources (Singh et al. 1983; Tait et al. 1994) can be produced by this reaction. CH$_3$I was not significantly different from the control in cultures of both N. seriata and P. glacialis and was only slightly higher in Nitzschia sp. CCMP 580 (R. Tokarczyk pers. comm.). Unless more rapid alternative pathways of CH$_3$I transformation to CH$_3$Cl exist, CH$_3$I cannot be the source of the CH$_3$Cl observed in our cultures.

DMSP (p-dimethylsulphoniopropionate), the biological precursor of DMS in seawater (Andreae 1990), can react with chloride ion to produce CH$_3$Cl (White 1982). An experiment that used high levels of DMSP (400 nM) in 0.2-μm filtered seawater (pH 8.0) sealed in a covered incubation vessel showed insignificant CH$_3$Cl production over a 1-week interval (Tait and Moore unpubl. results). One of the species we have cultured (P. tricornutum) has been shown to contain negligible amounts of DMSP in batch culture conditions (Keller et al. 1989). However, the greatest CH$_3$Cl production to date was found in vessels containing this species. It is unlikely that the chemical DMSP + chloride pathway of CH$_3$I formation can explain the CH$_3$Cl production observed in cultures of P. tricornutum.

Antibiotics (~1.0 mg ml$^{-1}$ streptomycin:2.0 mg ml$^{-1}$ penicillin) were added to the extended O. mobiliensis culture (Fig. 3) at 61 d. No further significant increase in either CH$_3$Cl or bacterial numbers was observed. Although this result suggests that bacteria may have contributed to CH$_3$Cl production after the death of all the phytoplankton cells, the leveling off may have resulted from exhaustion of an algal-produced substrate that reacted chemically to give CH$_3$Cl. We propose that the increase in CH$_3$Cl production after the death of all the phytoplankton cells occurs via precursors that are released by the phytoplankton and that are bacterially or chemically broken down to give CH$_3$Cl. Earlier in the
Fig. 3. Time series of CH₂Cl concentration in medium from a culture of *Odontella mobiliensis* (+) and a control vessel containing medium alone (●). Antibiotics (penicillin/streptomycin) were added at 61 d. Lower panel shows cell numbers.

Fig. 4. "Indirect" CH₂Cl production in culture medium from which *Odontella mobiliensis* cells were removed (3-μm Nuclepore). Error bars include uncertainty in the measured concentration from run-to-run precision at low concentrations of CH₂Cl (n = 9) and day-to-day variation of standards during the time series.

Due to the unknown effects of bacteria present in these cultures, our results must be defined as net CH₂Cl production. The sustained nature of CH₂Cl increase after the death of all the phytoplankton cells in the *O. mobiliensis* incubation suggests that in this case bacterial processes may contribute to net CH₂Cl production. However, evidence of microbial degradation of CH₂Cl has been reported. Fortuitous co-oxidation (see Stirling and Dalton 1979) of both CH₂Cl and CH₂Br has been shown to occur in cell-free suspensions of the broadly specific methane-oxygenase enzyme system (Stirling and Dalton 1979, 1980). There is also evidence (Hyman and Wood 1984) that ammonia mono-oxygenase from *Nitrosomonas europaea*, an autotrophic ammonia-oxidizing bacterium, is capable of performing similar co-oxidations.

The artificial conditions of culture (enclosure within a small volume, elevated nutrient levels, and high monospecific algal cell densities) may favor dominance of bacterial species and strains not abundant in natural marine populations. Both methane-oxidizing bacteria (Sieburth et al. 1993) and strains of *N. europaea* (Austin 1988) have been isolated from aerobic upper open-ocean waters. Microbial degradation of CH₂Cl and CH₂Br is possible within the aerobic upper layer of the oceans. Identification of species of bacteria present in the cultures will ultimately be necessary to determine whether bacterial processes occurring in the vessels are likely to occur in the environment. Phytoplankton might also be expected to behave differently in batch culture than in the natural environment. No fungi have been observed in any of the laboratory incubations.

The oceans are thought to be the source of ~80% of the flux of CH₂Cl to the troposphere (Crutzen et al. 1979; Rasmussen et al. 1980), but little is known about its dominant formation pathways in seawater. The unialgal culture experiments described here offer one means of investigating the nature of the marine biological source. Although the rates of halocarbon production observed under such conditions should be applied to the marine environment with extreme caution, a rough comparison has proved informative.

When scaled for the three orders of magnitude greater Chl *a* concentrations in culture compared with the marine environment, rates of CH₂Cl increase measured in our experiments (avg ~25 nmol m⁻³ d⁻¹) can account for <0.5% of the average net CH₂Cl required to sustain the
estimated sea-to-air flux. Therefore, although the production of CH$_3$Cl in phytoplankton culture has been clearly demonstrated, it is not yet clear how significant this production might be in the environment. The list of examined organisms is far from extensive and is dominated by diatoms. Despite this, a dependence of CH$_3$Cl over the eastern Pacific. J. Geophys. Res. 88: 3684-3690.


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References


Dissolved organic matter (DOM) in lake water can be divided into labile and recalcitrant moieties. Labile DOM by photochemical reactions (Kieber et al. 1989). Exposure to microbial degradation and transformation before reaching the lake and because, to a large extent, is based on recalcitrant support tissues of terrestrial vascular plants (Hobbie 1988). Nevertheless, high biomass of pelagic bacteria can be found in humic lakes, despite low or moderate autochthonous production of bacterial substrates. It has been suggested that this high biomass is due to extensive bacterial utilization of allochthonous DOM (Hessen 1985; Tranvik 1988).

The availability of recalcitrant DOM may be increased by photochemical reactions (Kieber et al. 1989). Exposure of humic water to short wavelength light bleaches the brown color, due to cleavage of large organic molecules and aromatic structures into smaller units (Strom and Miller 1978). Depletion of the stratospheric ozone layer (Gleason et al. 1993), resulting in increased UV-B irradiation, has stimulated studies of the role of UV-light in ecosystem processes. Considerable work has been done on the direct harmful effects of UV-B radiation on organisms in water (e.g. bacteria: Herndl et al. 1993; phytoplankton: Karentz et al. 1993), resulting in increased UV-B irradiation, has stimulated studies of the role of UV-light in ecosystem processes.

Our experiment shows that light exposure can enhance availability of natural lake DOM to bacteria, possibly through cleavage of macromolecules into smaller units, and may influence both carbon cycling and food webs in lake water.

Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter

Abstract—We tested the hypothesis that light, especially UV-B radiation, increases the availability of dissolved organic matter (DOM) to pelagic bacteria in lake water. Filtered (0.2 μm) and autoclaved humic lake water (water color 70 mg Pt liter−1, 12 mg dissolved organic C liter−1) was exposed to simulated sunlight (UV-B, 1.14 W m−2; UV-A, 3.87 W m−2; and PAR, 20 W m−2) for various periods of time (0–100 h). Irradiated water was then inoculated with a natural bacterial assemblage (0.6-μm filtered water) and bacterial yield was measured in the stationary phase of the resulting batch cultures. Both bacterial numbers and cell volumes increased (numbers by 65% and volumes as much as 360%) with increasing UV radiation, resulting in an almost sixfold increase in bacterial biomass. Our experiment shows that light exposure can enhance the availability of natural lake DOM to bacteria, possibly through cleavage of macromolecules into smaller units, and may influence both carbon cycling and food webs in lake water.

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Notes


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