

ENVIRONMENTAL MANAGEMENT SCIENCE PROGRAM

Stable Isotopic Investigations of *In Situ* Bioremediation of Chlorinated Organic Solvents

Progress Report for Work Performed in Fiscal Year 1997

Abstract

We have made significant progress in developing innovative methods for investigating the mechanism and extent of *in situ* bioremediation of chlorinated organic solvents. These methods use precise isotopic ratio measurements of chlorine and carbon in reactant and product species in laboratory experiments and in materials from field demonstration sites. Specific tasks completed during FY 1997 include: (1) refinement and publication of our new analytical method for precise determination of chlorine and carbon isotope ratios in chlorinated volatile organic compounds; (2) laboratory experiments involving biological degradation of chlorinated solvents in liquid cultures and soil columns; and (3) use of chlorine and carbon isotope ratios to investigate natural attenuation of trichloroethene at the Paducah Gaseous Diffusion Plant. This work can have immediate impact because it will provide the fundamental basis for a new and cost-effective means of evaluating and monitoring the effectiveness of *in situ* bioremediation schemes for chlorinated organic solvents in soils, vadose horizons, and groundwater plumes.

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Project Description

Background and Significance

Chlorinated volatile organic compounds (VOCs), including the common solvents perchloroethylene (PCE), trichloroethylene (TCE), carbon tetrachloride (CCl_4), chloroform (CHCl_3), and trichloroethane (TCA), are the most frequently detected groundwater contaminants in the United States (Fischer *et al.*, 1987) and occur at thousands of sites at U.S. Department of Energy (DOE) facilities (DOE, 1993). These compounds are designated as priority pollutants by the U.S. Environmental Protection Agency (EPA) (Keith and Telliard, 1979) and are known or suspected to be carcinogenic or mutagenic in humans. These compounds are readily transported by groundwater and are not reduced to acceptable concentrations for human consumption by most municipal water supply treatments; thus the compounds represent a significant hazard to a large portion of the human population. *In situ* bioremediation is an emerging technology that shows great promise for mitigation of chlorinated solvents at many of these contaminated sites.

Under its In Situ Technology Development Integrated Program, the EM-50 Office of Technology Development identified a need for a rapid method to detect *in situ* biodegradation products. A specific area of interest identified by EM-50 is the potential use of stableisotopic measurements of carbon and chlorine (i.e., $^{13}\text{C}/^{12}\text{C}$ and $^{37}\text{Cl}/^{35}\text{Cl}$) for performance evaluation of *in situ* bioremediation in the laboratory and the field. The premise of this approach is that stable isotope ratios may be used for tracing reactants and mineralization products (VOCs, carbon dioxide, microbial biomass, bound residues, and released chloride) and reaction pathways in environmental samples from contaminated groundwater plumes. Isotope ratios can provide evidence that *in situ* bioremediation is occurring in situations where chemical measurements provide insufficient or ambiguous data.

One of the most severe limitations of *in situ* bioremediation is the difficulty of proving when it is working or has worked (Madsen, 1991; National Research Council, 1993; EPA, 1994). This is particularly true for the highly volatile chlorinated aliphatics such as TCE, PCE, and CCl_4 . Current techniques for monitoring *in situ* bioremediation are unsatisfactory, and the work

proposed here will provide the fundamental basis for a new approach. The isotopic approach to performance evaluation of *in situ* bioremediation is a powerful alternative to conventional monitoring of VOC and CO, concentrations in contaminated groundwater plumes. The concentrations of VOCs and their degradation products in plumes may be difficult to relate to the efficiency of the remediation process because of dilution effects. This problem is mitigated to a large extent by measuring isotope ratios. If there is a significant isotopic fractionation between chlorinated VOCs and derivative chlorine-bearing products, then the fraction of chlorinated VOC that is dechlorinated can be inferred simply from the $^{37}\text{Cl}/^{35}\text{Cl}$ and/or $^{13}\text{C}/^{12}\text{C}$ isotope ratio of the residual chlorinated VOC. It is important to point out that there is currently no published information available on the magnitude of chlorine and carbon isotopic fractionation associated with biological degradation of chlorinated VOCs. We plan to help eliminate this important gap in our knowledge with the work being performed here.

The isotopic approach described here is relatively simple and can begin to be implemented immediately at the completion of this task. This isotopic approach can be applied to evaluate nonbiological remedial schemes for chlorinated solvents as well, such as those involving zero-valent metal catalysts or oxygen-producing compounds. This approach is less costly than many other chemical monitoring approaches and yields data that are more useful than most chemical measurements for establishing the mechanism and extent of *in situ* remediation.

Relevance to EMSP Goals

This work is relevant to EMSP goals because it will provide a new and cost-effective means of evaluating and monitoring the effectiveness of *in situ* bioremediation schemes. It will employ newly developed techniques to characterize isotopic fractionation (of chlorine and carbon) associated with biological degradation of chlorinated organic solvents in laboratory microcosms. These techniques and the data acquired by using them in laboratory studies will form the fundamental basis for quantitative assessment of the mechanisms, rates, and efficiencies of various *in situ* bioremediation schemes in field settings.

Summary of FY 1997 Progress

Task 1: Optimization of Analytical and Sampling Methods

Analytical Methods

We have developed a new and improved method for isotopic analysis of chlorine and carbon in chlorinated VOCs. An article describing this method (Holt et al., 1997) was published in the July 15, 1997 issue of the journal *Analytical Chemistry* (see Appendix 1). A patent application for this method was submitted to the U.S. Government Patent Office by the DOE Chicago Operations Office in August 1997.

In our analytical method, the chlorinated solvent is sealed in a Pyrex tube with CuO and combusted for two hours at 550°C to produce CuCl, CO₂, H₂O, and Cu₂O. The tube is opened for removal of CO₂, H₂O, and CuO. The CO₂ amount is measured and stored for determination of δ¹³C. Excess CH₃I is added to the combustion tube containing CuCl, and the tube is resealed for reaction of CH₃I with CuCl for two hours at 300°C to produce CH₃Cl and CuI. The CH₃Cl is purified cryogenically for mass spectrometric analysis. The precision of the method in laboratory measurements of reagent solvents is better than ±0.1‰ for both δ³⁷Cl and δ¹³C.

We have used this new method routinely for samples ranging in size from 2 to 250 μmol of chlorinated VOCs. We have found that, for samples smaller than about 2 μmol, purification by gas chromatography (GC) is required to remove trace amounts of CH₃I that are carried over from the cryogenic purification of CH₃Cl. We are in the process of modifying our mass spectrometer inlet system to allow analysis of small (< 2 μmol) samples on-line following GC purification. The cold-finger micro-inlet system on our mass spectrometer can analyze CH₃Cl samples as small as 0.1 μmol with acceptable precision.

Methods of Laboratory and Field Studies

We have devoted considerable effort to establish accurate techniques for sampling and isotopic analysis of chlorinated VOCs and their degradation products in both laboratory experiments and in samples from the field. These techniques can now be used to evaluate the

chlorine and carbon isotopic fractionations that occur during biodegradation of chlorinated VOCs. In this program we are performing some of the initial studies that will develop the use of stable isotope measurements of chlorine and carbon as a standard tool applicable for both fundamental mechanistic studies and field-based evaluation and monitoring studies of *in situ* bioremediation of chlorinated organic solvents.

For sampling chlorinated VOCs in headspace gas samples, we use cryogenic trapping procedures to concentrate and purify the VOCs. For sampling chlorinated VOCs in water, we use an on-line helium-stripping method, again with cryogenic trapping procedures. For collecting soil gases and groundwaters from wells, we use a procedure modified from Holt *et al.*, (1995), that involves evacuated glass flasks. For sampling groundwater from wells, we use a compressed-air bladder pump (“Well Wizard”) having Teflon-coated surfaces, with Teflon tubing for transport to the surface. Details of our sampling methods, which we are continually refining, will be published in journal articles along with research results.

Task 2: Laboratory Studies

Laboratory studies involving biodegradation of chlorinated VOCs in liquid cultures and soil columns have been in progress since early FY 1997. The results of these studies will be critical for helping to understand the mechanisms of biodegradation of chlorinated VOCs and for interpreting the isotopic data from field studies.

Our initial set of laboratory experiments involved dichloromethane (DCM) biodegradation in liquid cultures. Axenic cultures of MC8b, isolated from a DCM-degrading biofilter (Ergas *et al.*, 1994) were cultured under aerobic conditions in 50 mL of a chloride-free mineral medium in 254-mL bottles sealed with mininert valves. The bottles were shaken at 150 rpm at 22°C. Headspace DCM concentrations were monitored using a HP 5890 Series II gas chromatograph, and duplicate bottles were sacrificed for isotopic analysis of the residual DCM at various extents of degradation. Representative results are shown below for the variation in $\delta^{37}\text{Cl}$ of the residual DCM as a function of degradation (Fig. 1). These results are consistent with a Rayleigh-type isotopic fractionation having a fractionation factor of 3 to 5 per mil, with the

residual DCM being enriched in ^{37}Cl relative to the product chloride. This result indicates a preference by MC8b for detaching the more weakly bonded ^{35}Cl .

Two additional sets of liquid culture experiments currently underway involve TCE degradation by a consortium of aerobic toluene-degrading microbes and by a consortium of anaerobes. Because the TCE-degrading microbes cannot tolerate high TCE concentrations, we have scaled up the experiments in custom-designed 3-liter vessels.

Another set of liquid culture experiments is using microbes derived from water samples collected in contaminated wells at the Paducah Gaseous Diffusion Plant. We want to determine if this microbial community has potential for TCE degradation. A soil column experiment has been initiated using the aerobic MC8b microbe to degrade DCM. Once we have established the optimum procedure, we will set up other soil columns for experiments under various conditions to degrade a range of chlorinated VOCs.

Finally, we are collaborating with a group headed by Professor Teofilo Abrajano, Jr. at Memorial University, St. John's, NF in studying isotopic fractionation during reductive dehalogenation of chlorinated VOCs by metallic iron. A paper on this work will be presented at the Geological Society of America 1997 annual meeting in Salt Lake City, Utah (Dayan et al., 1997).

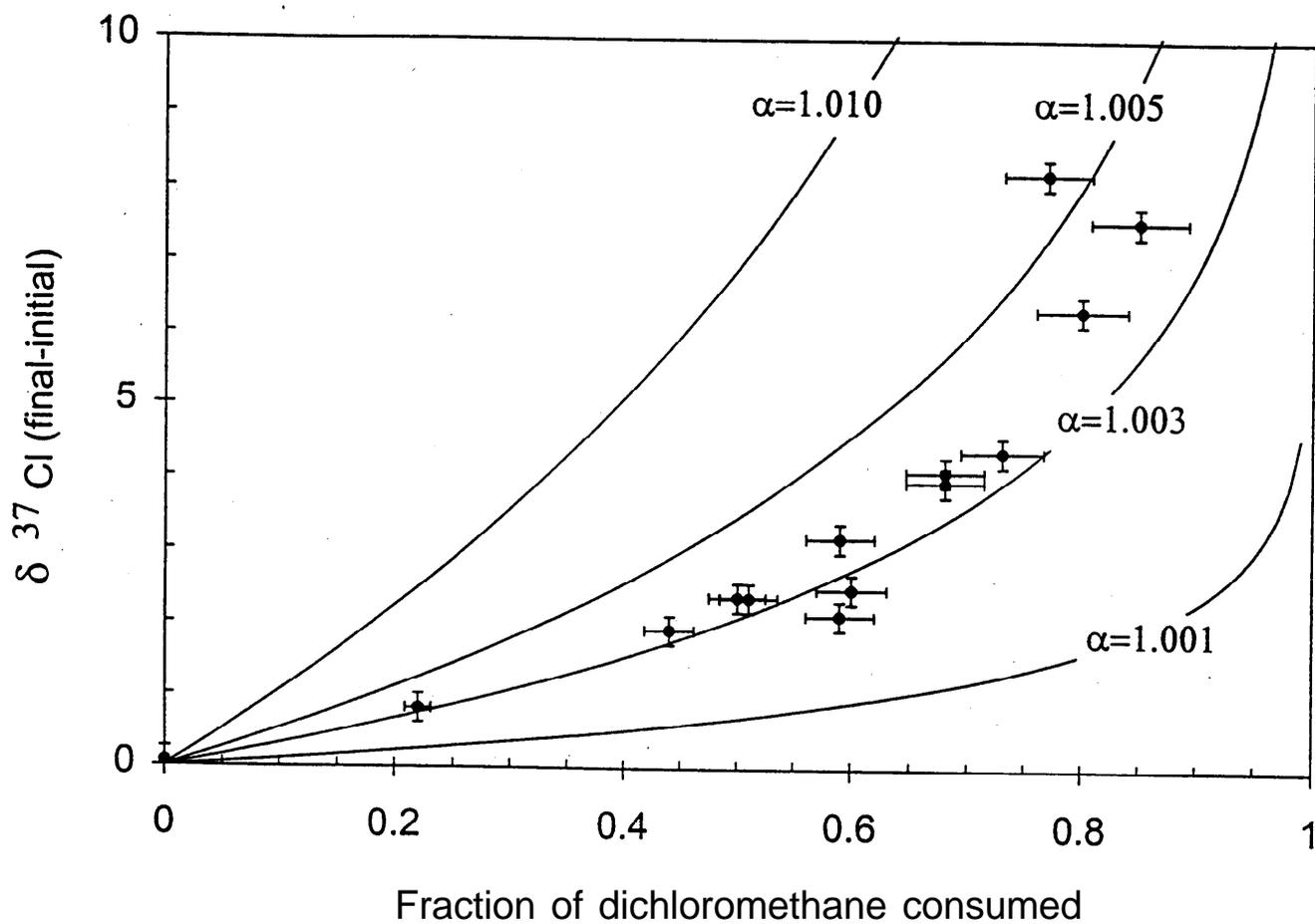


Fig. 1. Difference in $\delta^{37}\text{Cl}$ values of residual DCM as a function of the amount of DCM consumed by axenic MC8b culture. Data are consistent with Rayleigh-type fractionation with a DCM-chloride fractionation factor of 3 to 5 ‰. Microbial degradation clearly favors the detachment of ^{35}Cl from DCM.

Task 3: Field Studies

We have completed a reconnaissance study of natural attenuation of TCE at the Paducah Gaseous Diffusion Plant, Kentucky, in collaboration with Jay Clausen of Lockheed-Martin Environmental Services, Inc. (currently at Ogden Environmental and Energy Services, Westford, MA). We have also established contacts with site environmental management at the Portsmouth Gaseous Diffusion Plant, Ohio, as well as at the St. Joseph, Michigan site where natural attenuation of TCE has been well documented.

Paducah Gaseous Diffusion Plant

The first application of our method in the field was for a study of natural attenuation of TCE at the Paducah Gaseous Diffusion Plant (PGDP) in western Kentucky, USA (Sturchio et al., 1997). This site is contaminated with at least 2,000-3,000 liters of TCE. Two TCE-contaminated plumes extend northward in the regional gravel aquifer, toward the Ohio River, from residual TCE pool(s) on the PGDP property. Fifteen monitoring wells (including two uncontaminated background wells) were sampled during May 1997 for a preliminary isotopic investigation. The groundwater characteristics at the Paducah site are as follows: pH, 6.3-8.0; Eh, +120 to +280 mV; bicarbonate, 99-351 mg/L; chloride, 3-120 mg/L; sulfate, 5.5-115 mg/L; nitrate, <0.5-50 mg/L; total Fe, <0.3-7.0 mg/L; H₂S, <0.01-0.04 mg/L; ammonia, <0.1 mg/L; TCE, <0.001-180 mg/L; cis-1,2,-DCE, <0.001-0.130 mg/L. $\delta^{37}\text{Cl}$ values of TCE ranged from -1.0 to +2.0 ‰ relative to SMOC, and increase with decreasing TCE concentration (Fig. 2). There is apparently little or no difference in source composition of TCE in the two plumes.

There is good evidence for aerobic microbial respiration in the Paducah site groundwater, as evidence by a well defined negative correlation between dissolved O₂ and bicarbonate alkalinity. This, along with the observed Cl isotopic variation for TCE (Fig. 2), suggests the possibility of cometabolic aerobic degradation of TCE at this site. The Cl isotopic data for TCE at the Paducah site are not consistent with simple dilution, nor with fractionation by adsorption to aquifer solids.

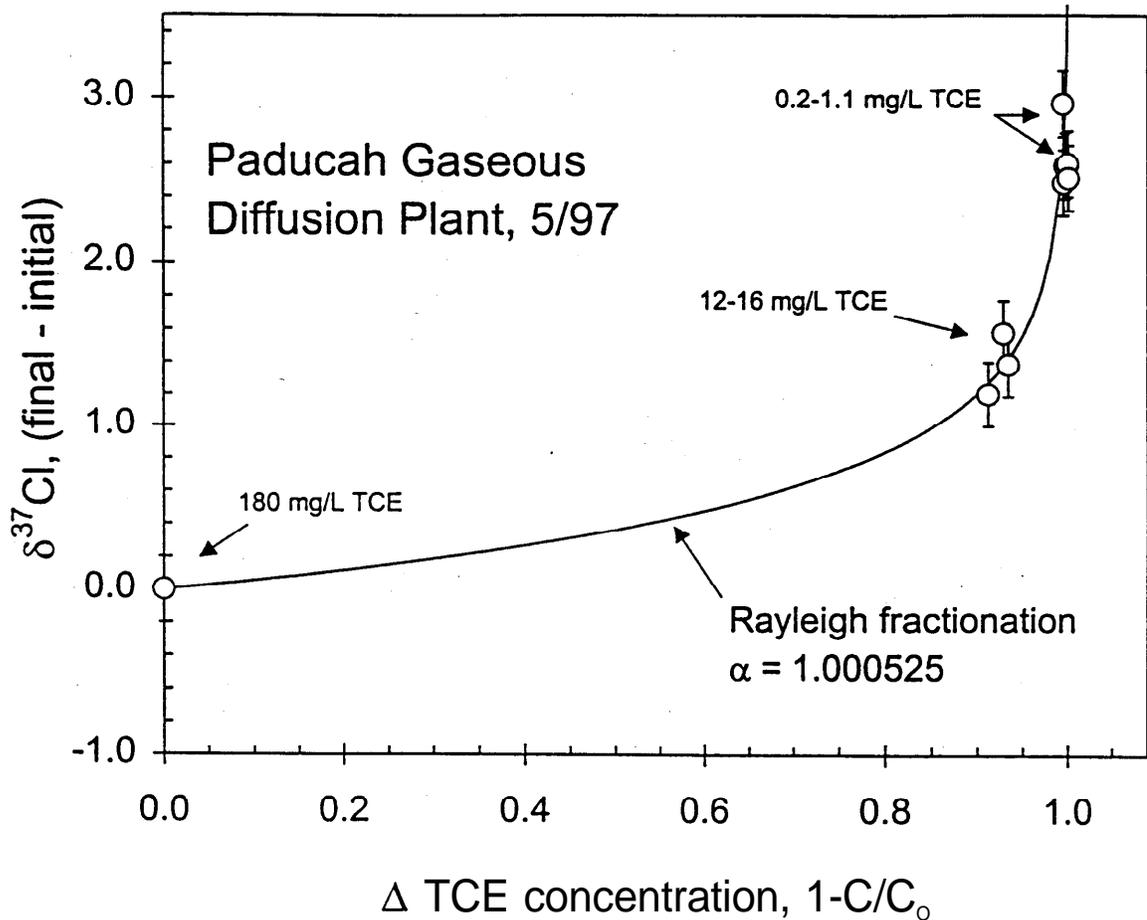


Fig. 2. Diagram showing change in $\delta^{37}\text{Cl}$ value of TCE as a function of concentration in contaminated groundwater plumes at the Paducah Gaseous Diffusion Plant, Kentucky. Concentration is plotted relative to an initial concentration of 180 mg/L above the separate-phase TCE pool at the source of the plume, so that the change in isotopic composition could easily be compared to that predicted by several different models. The data are consistent with a Rayleigh-type fractionation with a TCE-chloride fractionation factor of about 1.000525. Modeling of these data are in progress, taking into account our other isotopic data for dissolved inorganic carbon, inorganic chloride, and other geochemical parameters.

Plans for FY 1998

During FY 1998 we will proceed further with all three of our primary tasks: analytical and sampling methods development, laboratory experiments, and field studies. In the analytical and sampling methods development task, we plan to implement a continuous-flow capability for on-line Cl isotope ratio measurement of very small (i.e. <0.1 micromole) samples of CH₃Cl on our mass spectrometer. The ability to analyze such small samples is necessary for field studies of contaminated aquifers, where concentrations of chlorinated VOCs may be only in the ppb range. This will lead to the eventual ability to perform compound-specific Cl isotope analysis of mixtures of chlorinated VOCs. In the laboratory experiment task, we will continue our ongoing series of liquid culture and soil column experiments. We will also investigate the potential of Fe-bearing minerals for abiotic degradation of chlorinated VOCs. This mechanism of VOC degradation is suspected to be important, but has not yet been carefully investigated. If it is effective, it is important to know whether it has a characteristic isotopic fractionation that is distinct from that induced by microbial degradation. Finally, in the field studies task, we plan to study TCE plumes at the Portsmouth Gaseous Diffusion Plant (pending approval by plant management), as well as at the well characterized St. Joseph, Michigan site.

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Appendix 1

Conversion of Chlorinated Volatile Organic Compounds to Carbon Dioxide and Methyl Chloride for Isotopic Analysis of Carbon and Chlorine.

B. D. Holt, N. C. Sturchio, T. A. Abrajano, and L. J. Heraty
Analytical Chemistry **69**, 2727-2733 (1997).

Conversion of Chlorinated Volatile Organic Compounds to Carbon Dioxide and Methyl Chloride for Isotopic Analysis of Carbon and Chlorine

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A nonaqueous, high-temperature method is described for converting micromole quantities of chlorinated volatile organic compounds to CO₂ and CH₃Cl for C and Cl isotope ratio determinations. This method provides an improved analytical approach for using C and Cl isotope ratios in studies of the biodegradation of chlorinated volatile organic compounds in the environment. Conversion of reagent CH₃Cl to CO₂ + CuCl and then conversion of the CuCl back to CH₃Cl by the present method gives typical yields of 99 ± 1% for CO₂ and 91 ± 1% for CH₃Cl, both products of >99% purity. An offset of -0.23 ± 0.05‰ is observed between the ³⁷Cl/³⁵Cl ratios of product and initial CH₃Cl. Precision of the isotopic ratio measurements is better than ± 0.1‰ for a variety of chlorinated volatile organic compounds.

Chlorinated volatile organic compounds (VOCs), including the common solvents perchloroethylene, trichloroethylene, trichloroethane, and carbon tetrachloride, are among the most frequently detected groundwater contaminants in the United States. These compounds are known or suspected to be carcinogenic or mutagenic in humans. They are readily transported by groundwater and are not reduced to acceptable concentrations for human consumption by most municipal water supply treatments; thus, they represent a significant hazard to a large portion of the human population.

Measurements of the stable carbon and chlorine isotope ratios of chlorinated VOCs could be useful in tracing sources of these compounds and their breakdown products in the environment. This is particularly the case if these compounds have varied isotope ratios as a result of their manufacture and if biodegradation induces significant isotopic fractionation between residual reactants and products. It is currently difficult to determine the efficiency of natural attenuation or engineered bioremediation as a means of restoring soils and aquifers contaminated with chlorinated VOCs, but the possibility of using isotopic ratios to trace this process is now being explored. Some work has been done on measurement of stable chlorine isotopes in natural waters and rocks,¹² and recent work has been reported on a few selected chlorinated organic solvents.³

In preparation for our investigation of isotopic variations in VOCs, we have developed and are reporting here a new, non-aqueous, analytical method by which both CO₂ and CH₃Cl are produced from a single sample of a chlorinated VOC for isotopic analyses of chlorine and carbon. Currently used analytical methods require that CO₂ and CH₃Cl be prepared from a chlorinated compound by separate procedures. Van Warmerdam et al.³ prepared CO₂ by combusting samples of some chlorinated solvents with CuO at 550 °C by a published method.^{4,5} They then obtained CH₃Cl on separate samples of each solvent by first combusting in a Parr bomb (ASTM method D808-91) to produce chloride in carbonate solution (with yields in the range of 65-75%) and then converting the chloride to CH₃Cl, by a commonly used method of reacting AgCl with CH₃I.^{1,2,6,7} According to this method, the inorganic chloride is precipitated as AgCl, which is washed, filtered, and dried before being sealed in a reaction tube with excess CH₃I and heated for 2 days at 110-125 °C for reaction. The resulting CH₃Cl is separated from the CH₃I by gas chromatography before mass spectrometric determination of ³⁷Cl/³⁵Cl. Other methods of converting chlorinated VOCs to inorganic chloride for conversion to CH₃Cl involve reaction with sodium metal⁷ or lithium metal.⁸

By the method reported here, two reactions are carried out on the same sample of a chlorinated VOC. First the sample is heated with CuO for 2 h at 550 °C to form, among other products, CO₂ and CuCl. The CO₂ and the excess CuO are removed and the CuCl is then heated with excess CH₃I in the same reaction tube for 2 h at 300 °C to form CH₃Cl. The yields and purities of both the CO₂ and the CH₃Cl are suitable for mass spectrometric isotope ratio analyses without further treatment. The advantages of this method are as follows: (1) both CO₂ and CH₃Cl are produced from the same sample of material in the same reaction tube, (2) no aqueous chemistry is involved, (3) the two reactions require only 2 h each, (4) reproducible yields make it possible to use the method to assay materials for both percent C and percent Cl, and (5) no GC purification of the CH₃Cl is required.

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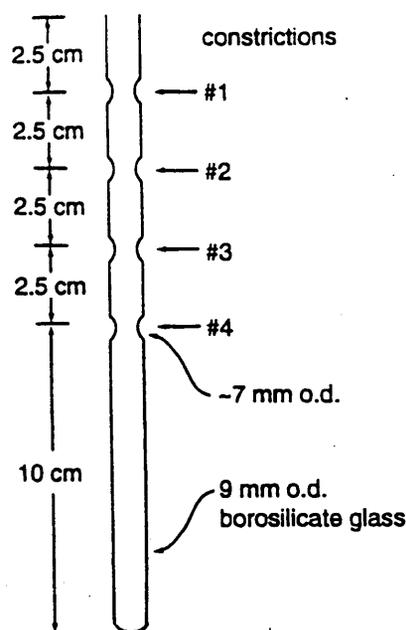


Figure 1. Diagram of combustion tube showing constrictions used for sealing and opening.

EXPERIMENTAL SECTION

Apparatus. (1) **Combustion Tube.** The design of the 20-cm, 9-mm-o.d., borosilicate glass combustion tube is shown in Figure 1. Four constrictions of ~7-mm o.d. are preformed in the tube as shown. Constrictions 1 and 3 are for sealing the tube, and constrictions 2 and 4 are for cracking open the tube.⁹

(2) **Vacuum Line.** Figure 2 is a diagram of the vacuum line. It is a grease-free all-glass line designed to sample, measure, and encapsulate gas or liquid samples and, to purify, measure, and encapsulate CO_2 and CH_3Cl for subsequent mass spectrometric analyses.

In the diagram, the Teflon valves are numbered upstream from stopcock S7 where the analytical line is connected to the main vacuum manifold. The main manifold, not shown, includes a thermocouple gauge, a liquid nitrogen (LN)-cooled cold trap, an oil diffusion pump, and a mechanical roughing pump. The purification cold trap assembly (including two traps, T1 and T2, monitored by gauge G1) and the submanifold assembly (monitored by gauge G2) are made of 16-mm tubing to allow adequate conductance of CH_3Cl vapor during cryogenic transfers. Cold trap T3, made of two loops of 8-mm tubing, is cooled with LN to protect the main manifold from waste chlorinated VOCs. This trap is demountable so that it can be flushed with air into a fume hood.

The dosing unit consists of a rubber septum at the end of a U-tube cold trap. Liquids introduced through the septum can be freed of entrapped air by evacuation through the cold trap. Storage receptacles (seal-off tubes or small gas sample bulbs) for CH_3Cl and CO_2 samples are attached through a Cajon coupling at V11. Other parts of the line are connected through Cajon couplings as indicated in the diagram. Electronic pressure measuring devices of comparable range may be used instead of the capillary mercury manometer.

(3) **CuCl Evaporation Furnace.** Figure 3 shows the arrangement for relocating the solidified CuCl condensate inside the combustion tube, after it is cooled to room temperature, to

avoid loss of CuCl when the tube is subsequently cracked open and resealed. Heat is applied by the tube furnace to distill the deposits of CuCl away from the CuO and from the glass walls in the zones of the constrictions. It is important that the CO_2 and H_2O vapors are first condensed in the LN-cooled butt end of the combustion tube, otherwise the CuCl will not distill efficiently and some of the CO_2 may thermally decompose to a black deposit of carbon char at the site of heating. The LN container consists of two stacked polystyrene-foam beverage cups bonded together with adhesive tape. The snugly fitting holes, through which the combustion tube penetrates the LN container, may be conveniently made to fit with an 8-mm cork borer and a rat tail file.

(4) **Mass Spectrometer.** Isotope ratio measurements of CO_2 and CH_3Cl were made using a VG Prism Series II dual-inlet triple-collector gas source mass spectrometer. Data acquisition was controlled by a personal computer using software provided with the instrument. Masses 44, 45, and 46 (CO_2^+ ions) and 50 and 52 (CH_3Cl^+ ions) were measured alternately in sample and standard gas. Major ion beam currents (at m/z 44 for CO_2 and m/z 50 for CH_3Cl) were maintained at 5.0×10^{-9} A.

Isotope ratios for C and Cl in samples were calculated by comparison with those in reference CO_2 and CH_3Cl that were calibrated to NBS-20 (Solenhofen Limestone) and Standard Mean Ocean Chloride (SMOC),² respectively. The absolute $^{13}\text{C}/^{12}\text{C}$ ratio in NBS-20 is 0.011 225 3 (given by Craig¹⁰) and the absolute $^{37}\text{Cl}/^{35}\text{Cl}$ ratio in seawater is 0.318 988 (given by Xiao et al.¹¹). Isotopic data in this paper are expressed in the conventional δ notation, where $\delta = 1000[(R_{\text{sample}}/R_{\text{standard}}) - 1]$ and R is the atom ratio $^{13}\text{C}/^{12}\text{C}$ or $^{37}\text{Cl}/^{35}\text{Cl}$. The resulting δ values are expressed in units of per mil (‰) deviation relative to the standard reference materials.

Procedure. (1) **Combustion of the Sample.** A group of combustion tubes (Figure 1), each containing 1 g of CuO (wire form, separated from fines on a 240- μm sieve), are ignited for 1 h at 550 °C. One of the tubes is attached to valve V21 (Figure 2) and, after evacuation of the entire vacuum line, flamed to free the CuO of adsorbed air and moisture.

If the chlorinated organic compound to be analyzed is a cryogenically transferable liquid, a sample is injected by syringe into the dosing unit through the rubber septum. By appropriate manipulation of the Teflon valves, the sample may be measured in the manometer, provided that the amount of sample is such that when completely vaporized in the manometer the pressure is less than the vapor pressure of the compound at room temperature. If the sample is a gas at room temperature, it may be possible to cryogenically transfer an aliquot from the sample container directly into the manometer for measurement. Sample sizes used in this work typically ranged from 10 to 70 μmol of CL.

The measured sample is cryogenically transferred to the combustion tube and sealed off at constriction 1 (Figure 1) with a methane-oxygen torch. After affixing an identification number on the glass tube, the tube is gently agitated in a horizontal position to evenly distribute the CuO into all sections of the combustion tube and then heated 2 h in a furnace at 550 °C.

(2) **Redistribution of the CuCl in the Combustion Tube.** With all the CuO particles evenly distributed between constrictions 1 and 3, the butt end of the combustion tube is inserted into the

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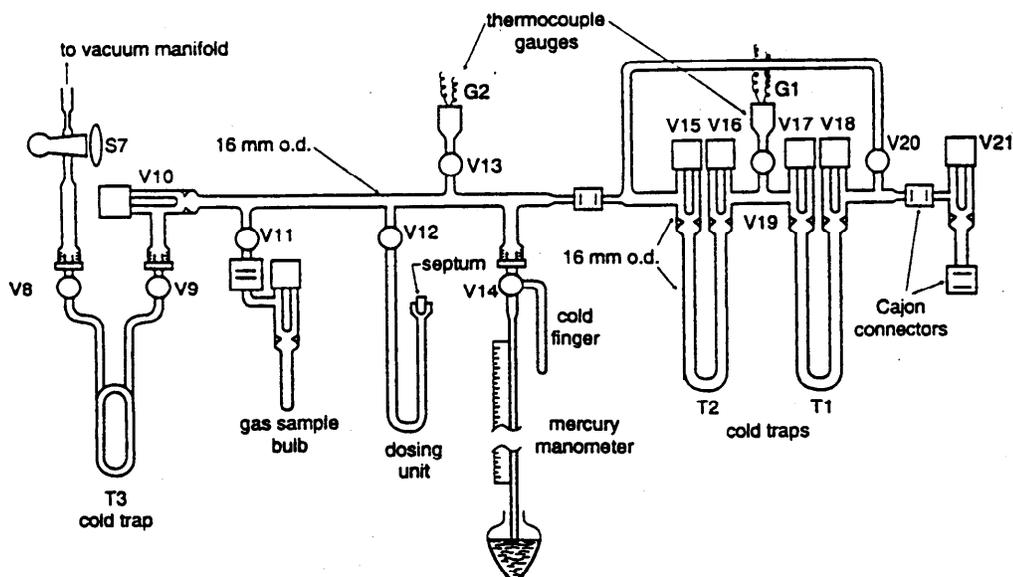


Figure 2. Diagram of vacuum line used for sample encapsulation and subsequent reaction product extraction and purification steps, with numbered valves (V), gauges (G), and traps (T).

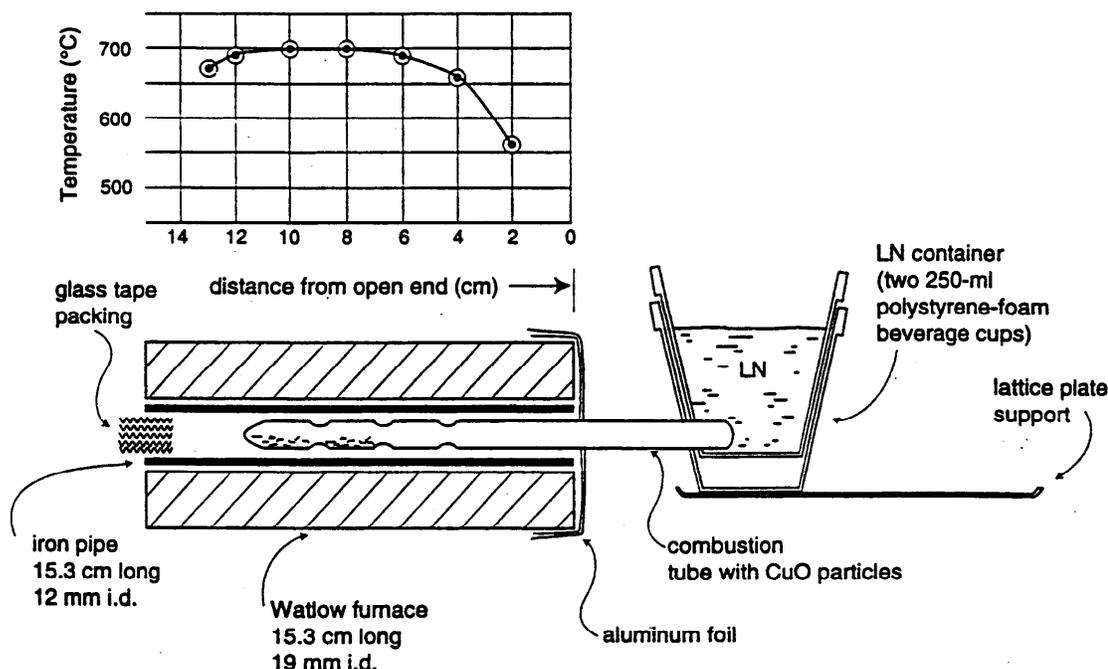


Figure 3. Diagram of apparatus for reevaporation of CuCl from the constrictions end of the combustion tube, showing temperature profile in the furnace at 700 °C.

snug fitting holes of the double-walled polystyrene-foam container (Figure 3) and LN is added to condense all CO_2 and H_2O . The tube is slid-into the furnace (midsection, -700°C) so that constriction 2 is ~ 9 cm from the open end of the 15-cm furnace. After 45 min, the tube is removed and allowed to reach room temperature with the CuO confined to the CuCl-free end.

(3) Removal of CO_2 and CuO from the Combustion Tube.

With the constrictions end of the tube pointing downward to maintain confinement of the CuO to the CuCl-free area, the tube is scratched at constriction 2 (for subsequent cracking open) and installed in a tube cracker. Valve V21 is rotated 180° at its Cajon joint so that after the tube cracker is attached, the scratched end of the combustion tube remains pointing downward.

With the Teflon valves arranged to collect H_2O in T1 [cooled by dry ice-acetone slush (DIA)] and CO_2 in T2 (cooled by LN),

the tube is cracked open at the scratched constriction (2), and noncondensable gases (NC) are pumped away.

The CO_2 is transferred from T2 to the manometer by moving the IN bath from T2 to the manometer cold finger and by moving the DIA from T1 to T2 (to retain any H_2O that may have passed through T1). After measurement, the CO_2 is cryogenically transferred to a gas sample container at V11 for storage for subsequent isotopic analysis. The tube cracker is removed from the line, and the end section of the glass tube containing the CuO is discarded.

(4) Reaction of the CuCl with CH_3I . The opened combustion tube is reattached at valve V21, which has been rotated 180° back to its original position (Figure 2). After evacuation of the line, $35\ \mu\text{L}$ of CH_3I is injected into the dosing unit, pumped free of air, cryogenically transferred to the combustion tube, and sealed

off at constriction 3. The sealed tube is placed in a furnace at 300 °C and heated for 2 h.

(5) Purification, Measurement, and Storage of the CH₃Cl. The glass tube is scratched at constriction 4 for subsequent opening, installed in the tube cracker, and reattached to the vacuum line at V21. After re-evacuation of the line, the valves and cold traps are arranged for collection of CH₃I in T1 [cooled by liquid-solid slush of *n*-pentane (-130 °C)] and CH₃Cl in T2 [cooled by LN (-196 °C)], and then the combustion tube [cooled by DIA (-79 °C)] is cracked open. The DIA cooling of the combustion tube enhances the cryogenic separation of the CH₃Cl from the excess CH₃I; without it the yield of CH₃Cl is adversely affected.

After 10 min, the DIA slush is removed from the combustion tube: then, after 10 min more, the CH₃Cl is transferred from T2 to the manometer by moving the LN bath from T2 to the manometer cold finger and the *n*-pentane slush from T1 to T2. This transfer of the *n*-pentane slush is necessary to entrap in T2 any CH₃I that was not completely retained in T1. Up to ~15 min (depending on the sample size) may be required for this transfer. During the transfer, the pressure of CH₃Cl in the line is indicated on gauge G2. At the end of the transfer (i.e., after the pressure reading of G2 has rapidly decreased to background level), the CH₃Cl is measured in the manometer and then cryogenically transferred to a gas sample container at V11 for subsequent isotopic analysis. The excess CH₃I is distilled back into the combustion tube for subsequent disposal to waste.

(6) Safety Considerations. There are several potential hazards associated with the use of this analytical method. First, the chlorinated organic compounds being analyzed, and the methyl iodide used in the procedure, may be carcinogenic and should thus be handled and disposed of accordingly. The use of a methane-oxygen torch for sealing glass tubes and the use of resistance furnaces for heating these tubes present burn hazards. The use of acetone and pentane in the cold traps, especially in the vicinity of the gas flame and hot furnace elements, poses a combustion hazard because of the low flash points of these compounds. The use of dry ice and liquid nitrogen carry the usual hazards of cryogenic materials and should be handled accordingly. The use of an all-glass vacuum line requires the use of glass components designed for such use to avoid the danger of implosion. Finally, care should be taken to ensure that sealed glass tubes do not contain enough volatile material to generate explosive pressure when heated; following the procedure as described here, we have not had any sealed tubes explode in our laboratory during hundreds of tests.

RESULTS AND DISCUSSION

Purities of CO₂ and CH₃Cl. Mass spectrometric analyses of the CO₂ and the CH₃Cl produced by the method typically showed both gases to be >99% pure when the final form of the procedure was followed. Earlier in the development of the method, when the CO₂ was cryogenically separated from H₂O by a one-step application of dry ice-acetone, the CO₂ product contained 1-2% H₂O. The adopted two-step purification procedure yields CO₂ with only traces of impurities. Likewise, in the case of CH₃Cl, the two-step purification procedure, using *n*-pentane slush to separate CH₃Cl from CH₃I, is necessary to reduce the CH₃I content in the product CH₃Cl to -0.2%. For the sample size range used in this work (10-70 μmol), further purification of the

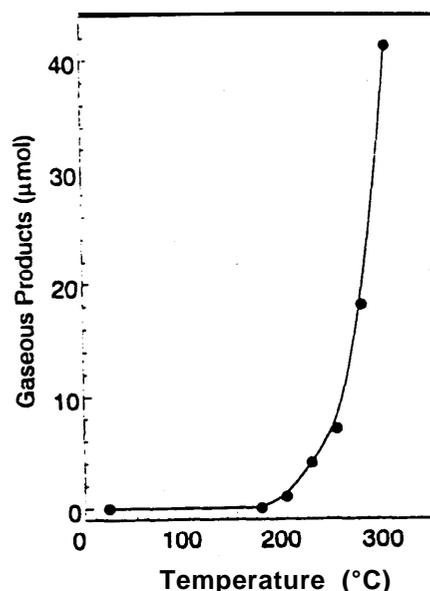


Figure 4. Reaction of CH₃I with CuO at various temperatures showing yield of undesired products.

CH₃Cl (e.g., by GC) was not necessary for subsequent mass spectrometric determination of δ³⁷Cl.

Separation of the excess CuO from the combustion tube, after the combustion reaction and before the reaction of the newly formed CuCl with CH₃I, was essential. If not removed, the CuO reacted with the CH₃I at 300 °C to produce intolerable amounts of gaseous contaminants, such as CO₂ and a mixture of unidentified organic products. Figure 4 shows the effect of heating 35-μL samples of CH₃I, each with 1 g of CuO at various temperatures.

Yields of CO₂ and CH₃Cl. The yields of CO₂ from the combustion of a variety of chlorinated organic compounds were typically 99 ± 1%. These results indicate complete oxidation of the organic carbon of the materials, as described by Sofer¹ in his use of borosilicate glass combustion tubes. We experienced very few problems with charring or tube breakage, as reported by Boutton et al.⁵ An important precaution for the prevention of charring was to evenly distribute the CuO in the borosilicate combustion tube before placing it horizontally in the furnace at 550 °C. Breakage of the glass tubes by thermal shock, after being sealed off by a methane-oxygen torch, was avoided by sealing at the appropriate performed constriction (Figure 1).

The overall yields of CH₃Cl from a variety of chlorinated organic compounds were typically 89 ± 4%. A search to find the source(s) of the deficiency in complete yield was centered on four phases of the analytical method. A discussion of the apparent effects of each of these four phases on the overall yield of CH₃Cl follows.

Effect of Separation and Purification of the CH₃Cl. This phase of the procedure was apparently responsible for -2% of the deficiency in yield. This is based on a test involving five samples in which amounts of tank CH₃Cl ranging from 9.1 to 69.2 μmol were each mixed with 35 μL of CH₃I in sealed tubes and subjected to the procedural steps of separation and purification. The average yield for these five samples was 98 ± 1%. When the condensates in the two cold traps, T1 and T2, were cryogenically returned to the combustion tube and the procedural steps repeated, the yield was 100 ± 1%. For normal use of the method, our preference was to forego a repetition of the purification

Table 1. Yields and $\delta^{37}\text{Cl}$ Values for CH_3Cl (from CuCl + CH_3I) Reaction under Various Experimental Conditions

| exptl conditions | n^a | yield (%) | n^b | $\delta^{37}\text{Cl}$ (‰) |
|---|-------|-----------|-------|----------------------------|
| CuCl unheated | 5 | 99 ± 1 | 2 | 2.1 ± 0.1 |
| CuCl evaporated | 4 | 98 ± 1 | 1 | 1.9 |
| CuCl heated with CuO (550 °C); evaporated | 9 | 90 ± 4 | 3 | 1.8 ± 0.1 |
| 0.5 μL of H_2O added; CuCl heated (550 °C); no CuO; evaporated | 6 | 85 ± 8 | 5 | 2.2 ± 0.2 |

^a Number of replicates. ^b Number of replicates; partial set of samples from yield test.

procedure and apply a correction factor of 1/0.98 to overall yield data.

One potential cause for poor yields of CH_3Cl in the purification procedure was incomplete cryogenic separation from the excess CH_3I during the transfer from the reaction tube to the manometer. It was important that the mixture be cooled with dry ice-acetone slush (-79 °C) before breaking open the combustion tube for the transfer. This allowed most of the more volatile CH_3Cl to separate from the CH_3I before entering the pentane slush cold trap (-130 °C) where co-condensation with relatively large amounts of CH_3I prolonged the residence time of the CH_3Cl in the trap.

Another potential cause for poor yields of CH_3Cl was failure to allow enough time for the cryogenic transfer from one cold trap to another. These transfers occur at a gas pressure approximating that of the vapor pressure of CH_3Cl at -130 °C. This low pressure requires that the inside diameter of the glass line be as large as practical and that adequate time be allowed for complete cryogenic transfer.

The purification procedure separated the CH_3Cl not only from excess CH_3I but also from products of thermal decomposition of small amounts of the CH_3I . These thermal decomposition products, from the reaction, $4\text{CH}_3\text{I} \leftrightarrow 2\text{CH}_4 + \text{I}_2 + \text{CH}_2\text{I}(\text{CH}_2\text{I})$, were easily identified. The I_2 was seen as a violet vapor in the heated reaction tube and as a bronze solution in the excess liquid CH_3I in the cooled reaction tube. The CH_4 was collected by a Toepler pump and identified by mass spectrometry. The $\text{CH}_2\text{I}(\text{CH}_2\text{I})$ was identified as a yellow solid of low vapor pressure on the inner walls of the vacuum line.

Effect of the Reaction of CuCl with CH_3I . The reaction, $\text{CuCl} + \text{CH}_3\text{I} \leftrightarrow \text{CH}_3\text{Cl} + \text{CuI}$, apparently proceeded to nearly complete yields of CH_3Cl within 2 h when CuCl was heated with a large excess of CH_3I in a sealed tube at 300 °C.

Table 1 gives results on four sets of samples of a supply of reagent grade CuCl. Samples (75 μmol) were weighed out in borosilicate glass capsules (8-mm length \times 4-mm o.d.) and dropped into the combustion tubes before forming the 7-mm constrictions in the tubes for sealing. The samples of the first set were untreated before the CH_3I reaction. The samples of the second set were not carried through the combustion step, but each was carried through the evaporation step before reaction with CH_3I . The samples of the third set were subjected to the entire procedure of the method, including combustion for 2 h at 550 °C and evaporation of the condensed CuCl in the combustion tube before reaction with CH_3I . Each of the samples of the fourth set were enclosed with 0.5 μL of H_2O before heating with CH_3I .

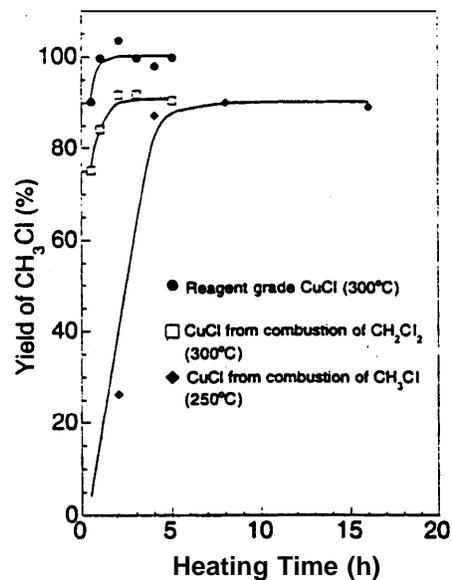


Figure 5. Yield of CH_3Cl vs heating time for the reaction $\text{CuCl} + \text{CH}_3\text{I} \leftrightarrow \text{CuI} + \text{CH}_3\text{Cl}$.

These data show that, with no prior treatment (and with a correction factor of 1/0.98 applied for loss in the CH_3Cl purification procedure described above), the average yield of CH_3Cl from the reaction of CuCl with CH_3I was >99%. This is comparable to the best that can be expected from the $\text{AgCl}-\text{CH}_3\text{I}$ reaction step of other methods.

Figure 5 shows the effects of temperature and the duration of heating on the yield of CH_3Cl . The yields of CH_3Cl are plotted vs heating times for reagent CuCl at 300 °C and for CuCl formed by the combustion of two chlorinated organic compounds at 300 and 250 °C. The optimum heating conditions for the reaction were 2 h at 300 °C. Other experiments showed that higher temperatures induced excessive thermal decomposition of the C&Cl and lower temperatures required unnecessarily long reaction times. Although the plotted data in Figure 5 indicate that the reaction at 300 °C was essentially complete within 1 h, our practice was to heat for 2 h.

The possibility of interference by I_2 in the reaction $\text{CH}_3\text{Cl} + \text{I}_2 \leftrightarrow \text{CH}_3\text{I} + \text{ICl}$ to significantly affect the yield of CH_3Cl was investigated. In one test, three samples of tank CH_3Cl (~75 μmol) were each heated with 35 μL of CH_3I for 2 h at 300 °C. No appreciable interference by the I_2 was evident in the measured recoveries of the CH_3Cl . In another test, the CuCl formed from combustion of tank CH_3Cl was equilibrated with CH_3I at lower temperatures (275, 250, and 225 °C) to test for any thermal shift in chemical equilibrium that might produce more or less ICl. No appreciable interference was indicated by the respective yields of CH_3Cl .

Effect of Combustion of the Sample. The essentially complete recoveries of CO_2 in combustion reactions (discussed above) show that no significant deficiency in yield of CH_3Cl can be attributed to incomplete combustion of the chlorinated organic compounds that were examined.

Effects of Physical and/or Chemical Changes in the CuCl. Since only ~2% of the observed average deficiency of -11% in the overall yield of CH_3Cl can be attributed to the CH_3Cl purification procedure, and 0% can be attributed to either the combustion reaction or the $\text{CuCl}-\text{CH}_3\text{I}$ reaction, the remaining yield deficiency of -9% is evidently related to what happens to

Table 2. Yields and $\delta^{37}\text{Cl}$ Offsets for CH_3I Samples Using Different CuCl Evaporation Procedures

| conditions for reevapn of CuCl | n ^a | CO ₂ | | CH ₃ Cl | |
|--|----------------|-----------------|---------------------------|------------------------|---|
| | | yield (%) | $\delta^{13}\text{C}$ (‰) | yield ^b (%) | $\Delta^{37}\text{Cl}$ ^c (‰) |
| vertical tube furnace at 790 °C CuO flame-heated at glass mp | 5 | 100 ± 1 | -58.02 ± 0.08 | 87 ± 1 | -0.45 ± 0.03 |
| horizontal tube furnace at 700 °C CuO not flame-heated | 6 | 99 ± 1 | nm ^d | 89 ± 1 | -0.23 ± 0.05 |
| horizontal tube furnace at 595 °C CuO not flame-heated | 2 | 99 ± 1 | nm ^d | 92 ± 1 | -0.26 ± 0.01 |

^a Number of replicates. ^b Uncorrected Cl yield. ^c $\Delta^{37}\text{Cl} = \delta^{37}\text{Cl}_{\text{product}} - \delta^{37}\text{Cl}_{\text{original}}$. ^d nm, not measured.

the CuCl after it is formed in the combustion reaction and before it is consumed by reaction with CH_3I .

Suspected sources of the incomplete yields in this phase of the method were as follows: (1) incomplete separation of adsorbed CuCl from the excess CuO before discarding the CuO, (2) incomplete reaction of the CuCl with CH_3I vapor because of a protective coating of newly formed CuI on unreacted CuCl, (3) formation of some CuCl_2 with the CuCl and (4) interaction of the CuCl with associated H_2O to form HCl.

Incomplete separation of adsorbed CuCl from the excess CuO was tested by three modified techniques. One was to heat at -795 °C for 10 min, using only the tube furnace (Figure 3). Another was to heat the tube with a methane-oxygen torch, in the vicinity of the CuO, to the softening point of the glass tube (~850 °C). And another was to heat even more intensely with the torch (~900 °C) for 5 min, resulting in partial thermal decomposition of the CuO to a sintered, brick-red mass of material (probably mostly Cu_2O). Comparative yield and PC1 data are given in Table 2 for some of these varied heating conditions. The more intense heating (e.g., at the melting point of glass) resulted in a small decrease in the average yield for CH_3Cl and a more significant decrease in $\delta^{37}\text{Cl}$.

The CuCl that evaporated from the heated end of the combustion tube in the tube furnace (Figure 1) tended to accumulate in bands of condensate just outside the furnace. To reduce the possibility that the formation of CuI coatings would significantly retard reaction of CH_3I in deeper layers of CuCl, these deposits were dispersed by reevaporation. The reevaporation brought no improvement in yield, showing that the effects of a protective coating of CuI over unreacted CuCl was not significant.

Formation of CuCl_2 by the reaction $2\text{CuCl} + \text{CuO} \leftrightarrow \text{CuCl}_2 + \text{Cu}_2\text{O}$ was also shown to not adversely affect the yield of CH_2Cl . In two experiments, using samples of reagent $\text{CuCl}\cdot 2\text{H}_2\text{O}$, the CuCl_2 was converted to CH_3Cl , by the reaction $2\text{CuCl}_2 + 4\text{CH}_3\text{I} \leftrightarrow 4\text{CH}_3\text{Cl} + 2\text{CuI} + \text{I}_2$, with yields of 95 and 97%.

Formation of HCl in addition to, and perhaps in equilibrium with, CH_3Cl was probably the major source of the loss in yield of Cl as CH_3Cl in this method. The reaction $2\text{CuCl} + \text{H}_2\text{O} \leftrightarrow \text{Cu}_2\text{O} + 2\text{HCl}$ is commonly thought to account for the deterioration of pure CuCl in moist air.¹² A test was made to identify HCl gas that might be pumped away as a noncondensable gas through traps T1 (-79 °C) and T2 (-196 °C) and out into the vacuum manifold. Three 65- μmol samples of reagent CuCl were each combined with -55 μmol of H_2O and sealed in combustion tubes. Two of the tubes were heated 2 h at 550 °C, according to the combustion procedure, but with no CuO present. Each of the three tubes was broken open directly into the inlet of a gas mass spectrometer.

(12) Mellor, J. W. *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*, John Wiley & Sons: New York 1961; Vol. III.

Table 3. Yields and Isotopic Data for Duplicate Analyses of Various Chlorinated Compounds

| compd | CO ₂ ^a | | CH ₃ Cl | |
|--|------------------------------|---------------------------|------------------------|----------------------------|
| | yield (%) | $\delta^{13}\text{C}$ (‰) | yield ^b (%) | $\delta^{37}\text{Cl}$ (‰) |
| CH ₃ Cl | 99 | -58.77 | 93 | -0.25 |
| | 98 | -58.03 | 91 | -0.26 |
| CH ₂ Cl ₂ | nm | -34.17 | 91 | +1.56 |
| | nm | -34.21 | 91 | +1.55 |
| CHCl ₃ | nm | -43.25 | 90 | -1.51 |
| | nm | -43.17 | 89 | -1.53 |
| CCl ₄ | 100 | -47.18 | 91 | -0.02 |
| | 100 | -47.25 | 92 | -0.01 |
| C ₂ H ₂ Cl ₂ | nm | -27.34 | 88 | +0.35 |
| | nm | -27.37 | 88 | +0.51 |
| C ₂ HCl ₃ | nm | -27.17 | 91 | -1.32 |
| | nm | -27.19 | 88 | -1.51 |
| C ₂ Cl ₄ | nm | -24.06 | 89 | +0.49 |
| | nm | -24.09 | 88 | +0.56 |
| C ₂ H ₃ Cl ₃ ^c | nm | -25.49 | 80 | -2.86 |
| | nm | -25.58 | 79 | -2.87 |
| CuCl | na | na | 99 | +2.13 |
| | na | na | 98 | +2.10 |
| CuCl ₂ ·H ₂ O | na | na | 95 | +0.44 |
| | na | na | 97 | +0.40 |

^a nm, not measured; na, not applicable. ^b Uncorrected Cl yield. ^c Solvent grade, containing 5% "preservative".

The measured concentration of HCl in the water vapor was -200 ppm. The Cl yields for the two $\text{CuCl} + \text{H}_2\text{O}$ mixtures that were carried through the combustion procedure, and for the unheated mixture of $\text{CuCl} + \text{H}_2\text{O}$, were all ~90%, compared to ~100% for CuCl that was neither heated nor exposed to added H_2O .

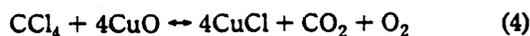
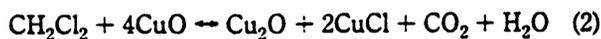
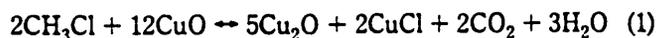
In Table 1, the yield and isotopic data for the fourth set of samples also show the effect of sealing 0.5 μL of H_2O in with -75 μmol of CuCl and carrying through the heating procedure without CuO . Results of this test indicate that the addition of water to the system, while causing significantly lower and more variable yields, also caused more variable $\delta^{37}\text{Cl}$ values.

Isotopic Precision of the Method. The isotopic precision of C and Cl isotope ratio measurements for various chlorinated VOCs analyzed using the new method can be assessed from the data in Tables 2 and 3. Specifically, the second row in Table 2 shows that the standard deviation for five replicate analyses of CH_3Cl by this method is $\pm 0.05\%$. The mean and standard deviation of the difference in δ values for duplicate analyses of the chlorinated VOCs listed in Table 3 are $0.05 \pm 0.03\%$ for $\delta^{13}\text{C}$ ($n = 7$) and $0.06 \pm 0.07\%$ ($n = 8$) for $\delta^{37}\text{Cl}$. We thus estimate that the precision is generally better than $\pm 0.1\%$ for both $\delta^{13}\text{C}$ and $\delta^{37}\text{Cl}$ in the analysis of the pure compounds.

APPLICATIONS OF THE METHOD

The method should be applicable to a wide range of chlorinated organic compounds. Some of the combustion reactions that

were examined in this study are



Some of the reaction products were visible on the inner walls of the combustion tube by microscopic examination. The CuCl appeared as finely divided deposits of colorless, solidified droplets. The Cu_2O was visible as sparsely distributed, ruby-red crystals. Expectedly, no red crystals were seen among the combustion products of CHCl_3 , CCl_4 , or C_2Cl_4 ; also, deposits of H_2O were visible on the tube walls (when cooled below 0 °C) for all of the above reactions except for CCl_4 and C_2Cl_4 .

Analysis of Tank CH_3Cl . Samples of tank CH_3Cl were converted to CO_2 + CuCl and then the CuCl was converted back to CH_3Cl by the procedure of the method to test for C and Cl yields and for alteration of the $\delta^{37}\text{Cl}$ value of the CH_3Cl by the procedure. Table 2- shows the results obtained for yields and isotopic values for both CO_2 and CH_3Cl under different conditions of heating, as previously discussed. Results show that by the following the procedure of the method, the average recovery of CH_3Cl was $91 \pm 1\%$ (after applying the 1/0.98 correction factor for the CH_3Cl purification step), and the $\delta^{37}\text{Cl}$ value was offset by $-0.23 \pm 0.05\%$.

Analyses of Other Chlorinated Organics. Table 3 lists results obtained on a variety of chlorinated VOCs that may be of interest in studies of such pollutants in soils and groundwaters. The method should be applicable to a wider range of such compounds, following the necessary extraction from environmen-

tal samples. The presence of excess water in the sample, however, can degrade the precision of the method, as indicated by the relatively larger standard deviation of $\delta^{37}\text{Cl}$ values in the fourth row in Table 1. Therefore, separation of water from environmental samples before analysis is important. Modifications of the method for environmental samples and for submicromolar sample sizes are now being developed in our laboratory.

The range in $\delta^{13}\text{C}$ values of the chlorinated organic compounds shown in Table 3 is **34%**, indicating that the initial isotopic ratios of such compounds can be useful for environmental tracing. The corresponding range in $\delta^{37}\text{Cl}$ values is only **4.5%**, and though it is much smaller than that of $\delta^{13}\text{C}$, it is large relative to the 0.1% precision of the method, indicating that initial Cl isotopic ratios of such compounds can be useful for environmental tracing. Isotopic fractionation caused by biological degradation of such compounds in the environment may extend the range of C and Cl isotopic variation significantly. While this type of fractionation may complicate the use of isotopic ratios for source apportionment, it provides a useful approach for helping to understand the extent of natural attenuation of chlorinated VOCs in contaminated groundwater plumes.

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