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Natural Formation of Chloroform and Brominated Trihalomethanes in Soil

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We studied the occurrence of halogenated organic compounds in soil air of rural areas. Chloroform appeared to occur in elevated concentrations compared to those in atmospheric air, while the concentrations of other chlorinated solvents were almost equal or lower than those in atmospheric air. We report conclusive evidence that chloroform is naturally produced from in situ $Na^{37}Cl$ enrichment field studies in soil top layers. The concentration of chloroform in soil air increased in deeper soil layers, but spiking of these soil layers by $Na^{37}Cl$ did not result in the formation of chloroform enriched with ^{37}Cl. Bromodichloromethane shows similar concentration gradients in soil air as does chloroform. It seems also to be formed naturally in soil, even although this could not be confirmed by the $Na^{37}Cl$ enrichment field studies, because of the low concentration levels encountered. No detectable concentrations of chlorodibromomethane and bromoform in soil air were observed. In situ enrichment of a soil top layer by KBr showed that soil has the potential to form chlorodibromomethane and bromoform naturally. The formation mechanisms of the trihalomethanes are discussed, and a hypothesis is given to explain the natural formation in the soil top layer and the concentration gradients in soil air.

Introduction

Chloroform is an ubiquitous environmental compound. Because it is very volatile any emission of chloroform will end up in the troposphere where its average concentration is about 0.1 ng/L. Chloroform is an enigmatic compound with large missing global sources (cf. (*1*) and (*2*)). Chloroform is considered as a possible important contributor of reactive chlorine in the troposphere and is therefore one of the subjects in the Reactive Chlorine Emission Inventory (*3*).

Apart from emission due to industrial processes, chloroform is produced during anthropogenic activities such as chlorination of drinking water, swimming water, and cooling water, pulp and paper bleaching, and traffic exhaust (*4*). The formation of chloroform from atmospheric degradation of the man-made solvents trichloroethene and 1,1,1-trichloroethane (*4*) can also be considered as an anthropogenic source.

In addition to these anthropogenic sources, chloroform has been found to be produced from natural sources. Rudolph et al. (*5*) found chloroform emission as a result of savannah fires. Although the real origin of the production of chloroform during biomass burning can be disputed, if biomass fires are ignited by a natural source, e.g., lightning, chloroform is formed purely naturally. Chloroform has also been detected in emissions of volcanoes (*6*), hydrothermal sources (*7*), and salt mines (*8*). The mechanism of chloroform formation remains unknown, but Isidorov speculates that chloride is transformed into reactive chlorine species by lithospherical processes and subsequent chlorination of organic material which is present.

Purely natural sources of chloroform have been identified by McConnell and Fenical (*9*) and Nightingale et al. (*10*), who reported the production of chloroform by algae and sea weeds, respectively. Another natural source of chloroform was identified by Khalil et al. (*16*) who found concentrations of chloroform up to 1800 ng/L in air inside termite mounds in Australia. However, it is unclear if chloroform is formed by the termites themselves or by other organisms that also live in the mounds. As an example, fungi can also be suspected because they are cultivated by some termite species as food for fertile adults and young larvae. Some soil fungi have been demonstrated to produce chloroform *de novo* (*11*).

That soil, in general, is a probable source of chloroform appeared from the measurements by Frank and Frank (*12*) and Hoekstra and De Leer (*13*) who observed that the concentration of chloroform in soil air is 5−620-fold higher than in atmospheric air. The concentrations of all other volatile chlorinated solvents which have comparable physical-chemical properties were found to be equal or lower in soil air than in atmospheric air.

In this paper, we provide conclusive evidence that chloroform is produced naturally in soil. The natural chlorination of organic material in soil can become a combined process of chlorination plus bromination if bromide is present in soil, as will probably be true in, e.g., coastal areas. The natural formation of bromoform in soil can be important because of the potential role attributed to bromoform as one of the regulative species of the ozone layer thickness (*14*). We report the formation of bromoform and chlorodibromomethane as a result of in situ bromide addition in soil.

Experimental Section

Sampling. Since the vegetation determines the composition of the litter and humic layer and thus possibly the formation of chloroform, two rural forests sites in The Netherlands were selected on the basis relatively undisturbed history. The Speulderbos near Apeldoorn has been a forest for more than 150 years. Two areas were selected: area no. 11O, a Douglas forest which was replanted in 1959 without using fertilizers, is also used in the Dutch acid rain and soil acidification research program, whereas area no. 6B is a beech forest which was replanted in 1835. The dunes of Wassenaar were selected because of the possibility of a direct influence of sea spray and, as a consequence, bromide input into the soil. As part of a sampling program of the University of Denmark *(15)*, samples were also taken in a spruce forest at Klosterhede (Denmark). In the city of Delft an urban site was chosen that is situated in the main plume of the highly industrialized

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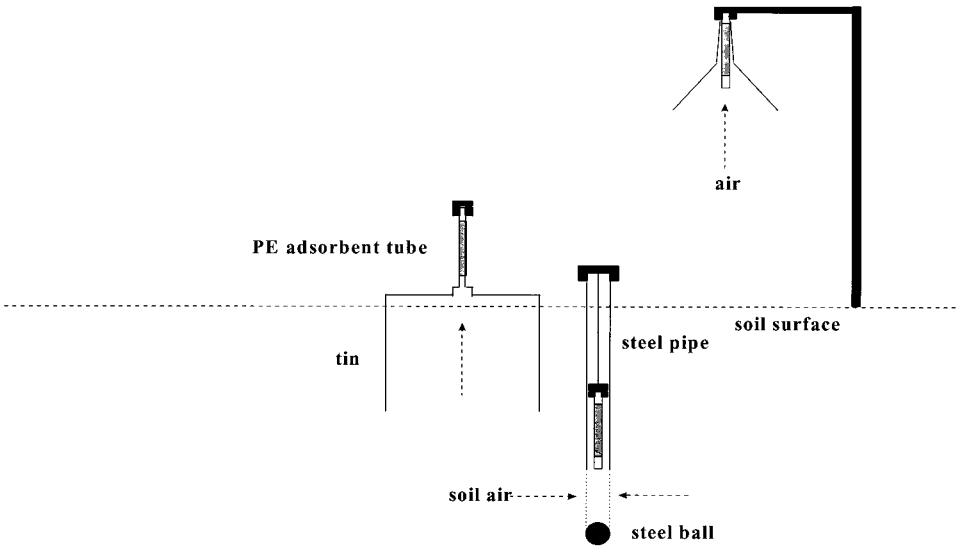

FIGURE 1. Soil air and atmospheric air sampling. For further explanation, see text.

area of Rotterdam; an area covered with wood chips was sampled.

Atmospheric air was sampled from about 5−10 cm above the soil surface by passive sampling (*16*) using PE (Perkin-Elmer) adsorbent tubes filled with 250 mg of Tenax-GR (Buchem; 60−80 mesh) during 2−4 weeks. The opening of the tubes was protected against rain by putting the tubes in the stem of an upside-down funnel, with the opening of the tube at the bottom (Figure 1). Soil air of the soil top layer was sampled by enclosures (Ø = 12.5 cm, h = 20 cm) on which PE diffusive samplers were mounted by means of Swagelok bulk-head unions. The enclosures were carefully put into the soil in such a way that the soil structure was not disturbed, and a headspace of about 5 cm was present between the soil surface and the enclosure (Figure 1).

Soil air below the top soil layer was sampled by hammering stainless steel pipes (type 304; $Ø_{in}$ = 12 mm; $Ø_{out}$ = 15 mm) into the soil (Figure 1) which could be done manually as deep as about 2 m. To prevent soil entering the pipe during hammering, a stainless steel ball was put in front of the pipe. The pipe was put into the soil about 10 cm deeper than its final position in order to create sufficient space for the volatile compounds to diffuse into. After the pipe had been put into position, a snugly fitting PE adsorbent tube was inserted in the pipe with the open end being in the lower part of the tube (see Figure 1). The top end of the tube was tightened by a plastic plug. Soil air was sampled in the passive mode during about 2 weeks.

Chloride-37 Isotope and Bromide Experiments. To obtain evidence on natural formation of chlorinated compounds in soil, the soil surface (123 cm^2) was spiked with a solution of sodium chloride-37 (95% $Na^{37}Cl$; CIL CHLM-1225) in a concentration range of 1−10 g $Na^{37}Cl$ per m^2. Ten milliliters of the concerning solution was spread dropwise by a syringe. Ten milliliters of a 27 g/L solution of potassium bromide (KBr pA; Merck 4905) was put on the soil to obtain evidence concerning the formation of brominated compounds in soil. The spiked area was covered by the soil lid, and the compounds were sampled from the soil air by passive sampling. In the control experiments no NaCl was added. The additional value of the enclosure in the enrichment studies was that it prevented the leaching of $Na^{37}Cl$ and KBr with rainwater to deeper soil layers.

Analysis. The adsorbent tubes were analyzed by mounting them on an automatic thermal desorption unit (Perkin-Elmer ATD-400) which was connected to a gas chromatograph (Varian 3400) and an ion-trap mass spectrometer (Finnigan Mat MD 500). The adsorbed compounds were desorbed with helium gas at 300 °C for 10 min, and they were trapped at −100 °C in a cold trap containing Tenax-GR. For injection, the cold trap was heated to 300 °C for 5 min. Neither the inlet nor the outlet split of the cold trap was used. The temperatures of the valve and the transfer line to the gas chromatograph were 180 and 200 °C, respectively.

The capillary column (DB-5 or CP−Cil 8 CB; l = 60 m; $Ø_{out}$ = 0.25 mm; film thickness = 0.25 μm) was programmed as follows: 0 °C (3 min hold) to 90 °C at 3 °C/min and next to 280 °C at 10 °C/min with a final hold of 2 min. The pressure on the capillary column was set to 35 psi. The transfer line to the ion-trap mass spectrometer was held at 210 °C. The ion-trap mass spectrometer used a mass range of m/z 46−280.

Quality Assurance. After thermal conditioning, one of every series of 20 adsorbent tubes was tested for blank levels. Both benzene and toluene showed blank values of 0.5 ng at maximum. Travel blanks were as clean as the adsorbent tubes just after thermal conditioning.

All the analytes were identified from their retention time and mass spectrum. The amount of analyte which had been adsorbed on the adsorbent tube was calculated from standard tubes which were analyzed together with a series of samples. The standard tubes contained tri- (83) and tetrachloromethane (117), tri- (130) and tetrachloroethene (166), 1,1,1-trichloroethane (97), benzene (78), and toluene (91) with their characteristic m/z values indicated between brackets. The standards were prepared in the gas generation laboratory of NMi. The calibration curve was linear ($R^2 \geq 0.99$) in the range of 5−100 ng for the aromatics and 0.5−25 ng for the chlorinated compounds at their characteristic m/z values as indicated above. The detection limits of the aromatics were about 0.01 ng and the detection limits of the chlorinated compounds about 0.02 ng. The amounts of bromoform (173), chlorodibromomethane (129), and bromodichloromethane (83) were calculated from the response factor of chloroform. The concentrations of the compounds which were sampled by passive sampling were calculated from the amounts adsorbed on the adsorbent tubes by using uptake rates from Peters et al. (*16*). Since the uptake rate of chloroform is not known for sampling periods of 2−4 weeks but only for 5 h (2.2 ng/ppm/min), it was compared with those of very similar compounds such as 1,1,1-trichloroethane and tetrachloromethane. According to Peters et al., the uptake rates of 1,1,1-trichloroethane and tetrachloromethane in the 2−4 week period are the same to within 5%; we therefore

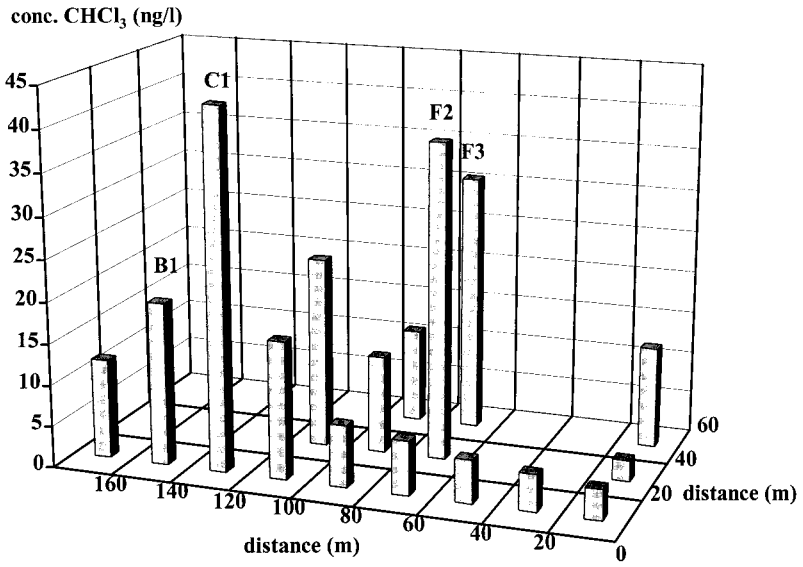

FIGURE 2. The variability of the concentration of chloroform in soil air from a Douglas forest in Speulderbos sampled by enclosures. B1, C1, F2, and F3 are the numbers of the sampling sites discussed in the text.

extrapolate the uptake rate of chloroform to this value. Compounds such as tri- and tetrachloroethene, benzene, and toluene showed an uptake behavior different from that of 1,1,1-trichloroethane and tetrachloromethane in the 2−4 week period.

Results

Isotopic Experiments in the Field. To provide conclusive evidence for the natural formation of chloroform from inorganic chloride in soil we enriched the soil surface of several field sites by adding $Na^{37}Cl$. If the hypothesis is true, the chlorine isotope fraction in the enrichment experiments should differ from the natural isotope fraction of ^{37}Cl in chloroform, i.e., 0.24. After sampling and analysis, the isotope fraction of ^{37}Cl in the mass fragment $-CHCl_2$ ($m/z = 83, 85, 87$) of chloroform was determined. No interference from mass fragments of other compounds was observed for any of the selected m/z values. Initial field experiments with ^{37}Cl were carried out at a location covered with wood chips where 1 g of $Na^{37}Cl$ per m^2 was added. Chloroform was formed from $Na^{37}Cl$ within 15 days, and the isotope fraction of ^{37}Cl in chloroform remained essentially constant at 0.29 during at least 1 year. The percentage of chloroform formed from $Na^{37}Cl$ had a maximum of 17 wt %. In all control experiments we observed the natural isotope fraction of ^{37}Cl, i.e., 0.24 ± 0.005, in chloroform.

The possibility of chlorine exchange between inorganic chloride and the chlorine atoms of chloroform was studied in experiments in which milli-Q water (9 mL) and wet sterilized soil (3 g of soil + 9 mL of milli-Q) were spiked with chloroform (0.84 nmol) and $Na^{37}Cl$ (0.16 mmol). The concentration of ^{37}Cl in the experiment with soil was about 1000-fold higher than that of Cl found in the field, while the chloroform concentrations were comparable. After 8 days in daylight, no formation of chloroform from $Na^{37}Cl$ was observed in either experiment.

As the next step, experiments were carried out in a Douglas forest in Speulderbos. The spatial variability of the concentration of chloroform in soil air was studied first, and a rather wide range, i.e., 5−40 ng/L, was found as is illustrated in Figure 2. The used passive sampling method with enclosures did not allow us to conclude at which depth the found concentrations were present since the formation of chloroform causes an accumulation in the headspace and a change in the chloroform concentration gradient in soil. From concentration gradient measurements (see below) it appears that our enclosure sampling method represents the concentration of chloroform at a depth of 40−160 cm.

Four sites, i.e., B1, C1, F2, F3 of Figure 2, were selected to be spiked in situ with $Na^{37}Cl$ because the sites with the largest concentrations of chloroform in soil air were expected to be the most active sites. Here, higher amounts of ^{37}Cl (10 g of $Na^{37}Cl$ per m^2) were supplied in order to effect a larger change in the isotope fraction of ^{37}Cl. Somewhat surprisingly, only small amounts of chloroform formed from $Na^{37}Cl$ were found at site B1 during the first 2 weeks. However, the isotope fraction of ^{37}Cl in chloroform increased dramatically to about 0.40 at site B1 when the soil layer was slightly disturbed by simply taking out the enclosure and replacing it (Figure 3). About 30 wt % of the sampled chloroform was calculated to be formed from $Na^{37}Cl$ (see Table 1). Addition of $Na^{37}Cl$ did not affect the concentration of chloroform, i.e., 14 ± 2 ng/L before and after addition but only the isotope fraction of ^{37}Cl in chloroform. At the other sampling sites, C1, F2, and F3, the formation of chloroform from $Na^{37}Cl$ also started only after disturbing the soil slightly, but the isotope fraction of ^{37}Cl reached a maximum of 0.28 only which is lower as at site B1. The phenomenon that the formation of chloroform from $Na^{37}Cl$ started after slightly disturbing the soil was also observed in soil air from the beech forest. The striking mutual differences cannot be explained yet. The proper method of application may well play a role: three-dimensional distribution of the $Na^{37}Cl$ solution versus heterogeneity of bioactivity in soil.

Place of Chloroform Formation in Soil. Table 2 shows the concentration gradient of chloroform in soil air with the soil depth at site B1 in the Douglas forest. The maximum concentration of chloroform is found below 20 cm in depth and remains almost stable at a concentration of 20−30 ng/L down to 160 cm. Two of the three concentration gradients show a maximum of 36 ng/L at 120 cm depth which suggests that this layer may be a source for chloroform in soil. At two other sampling sites from area B1 in the Douglas forest and at two sampling sites in the beech forest a lower maximum concentration of chloroform of 4−10 and 2−4 ng/L, respectively, was observed in the soil layers of 40 and 80 cm. The concentration of chloroform in the spruce forest in Denmark measured by active sampling 1 L of soil air on an adsorbent tube at a rate of 50−100 mL/min increases linearly up to about 55 ng/L at a depth of 40 cm which was measured at

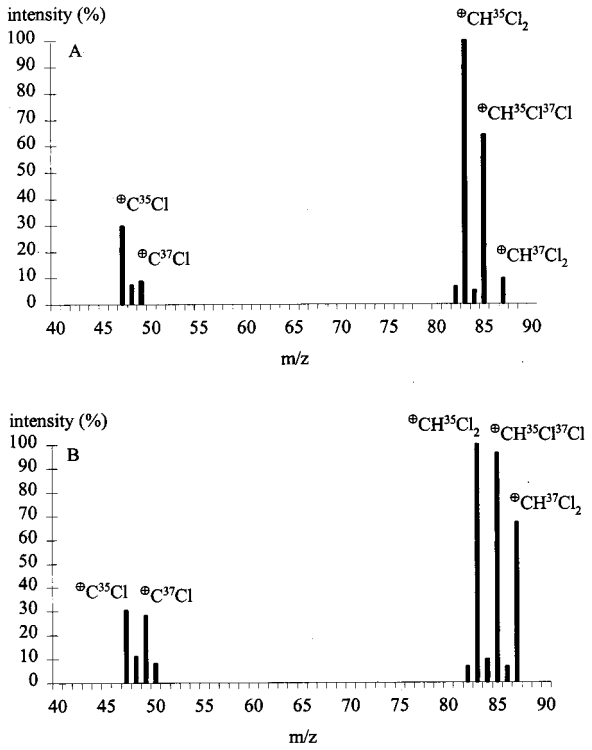

FIGURE 3. Mass spectra of chloroform in soil air from site B1 in Speulderbos: (A) a control area and (B) an area enriched with $Na^{37}Cl$.

two sampling sites. The concentrations of tetrachloromethane and other chlorinated C_2 organic compounds are more or less independent of the soil depth at all sampling sites.

The concentration gradient of chloroform in soil suggests that most chloroform is not produced in the soil top layer. To study the possible natural formation of chloroform in deeper soil layers, soil layers at site B1 in the Douglas forest were spiked via sampling pipes with 1 mL of a solution of 10 g of $Na^{37}Cl$/L. No formation of chloroform enriched with ^{37}Cl was found.

Brominated Trihalomethanes. Bromodichloromethane was detected in nearly all the samples of soil air in the Douglas forest in a concentration of 0.019 ± 0.009 ng/L but was not detected in atmospheric air. The concentration of bromodichloromethane is higher in deeper soil layers and has a maximum at 120 cm depth (2 out of 3) as also was observed for chloroform (see Table 2). The concentration gradient strongly suggests that the soil of the Douglas forest emits bromodichloromethane. The isotope fraction of ^{37}Cl in bromodichloromethane could not be determined with an acceptable standard deviation, because the concentration was close to the detection limit of 0.005 ng/L. As a consequence, the $Na^{37}Cl$ addition experiment discussed above could not be used to demonstrate whether bromodichloromethane was also formed naturally from $Na^{37}Cl$. The concentrations of chloroform and bromodichloromethane did not change notably after the addition of bromide to the soil. Comparable results were obtained by Peters et al. (*17*) in experiments on the chlorination of drinking water. The addition of bromide in their experiments resulted in the formation of large amounts of bromoform and smaller amounts of chlorodibromomethane, while the amounts of chloroform and bromodichloromethane formed were slightly lower than in the absence of bromide.

Bromoform was detected only in atmospheric air, with concentrations of 0.018 ± 0.006 ng/L, and chlorodibromomethane was not detected in either atmosphere or soil air of the Douglas forest. After the addition of a potassium bromide solution to soil of the Douglas forest, the formation of bromoform (0.14 ± 0.08 ng/L) and chlorodibromomethane (at the detection limit of 0.005 ng/L) was observed.

One sampling site in a pine forest in the dunes near the North Sea was chosen in order to see if bromide in sea spray can cause an increased formation of brominated trihalomethanes in soil of coastal areas. No chlorodibromomethane and bromoform were detected. The concentration of chloroform in soil air was only 2−3-fold higher than in atmospheric air which strongly indicates that no or only low natural chlorination activity was present in soil at the chosen sampling site. Further studies should establish if bromide in sea spray can cause natural formation of chlorodibromomethane and bromoform in coastal areas with higher chlorination activity in soil.

Discussion

The natural formation of chloroform in soil can be explained by the formation of reactive chlorine species, such as hypochlorous acid, from chloride and hydrogen peroxide by a chloroperoxidase (CPO)-mediated reaction [eq 1] (*18*). Next, the reactive chlorine species chlorinate the humic material in soil in a nonspecific way [eq 2]. If bromide is present, reactive chlorine species can react rapidly with bromide to form hypobromous acid [eq 3] which can brominate organic compounds similar to eq 2.

$$H_2O_2 + H^+ + Cl^- \xrightarrow{CPO} HOCl + H_2O \quad (1)$$

$$\text{humic material} + HOCl \rightarrow \text{chlorinated humic material} + CHCl_3 + CCl_3COOH + \ldots \quad (2)$$

$$HOCl + Br^- \rightarrow HOBr + Cl^- \quad (3)$$

Walter and Ballschmiter (*19*) studied the formation of chlorinated products from simple organic compounds such as acetone, propionic acid, and citric acid in the presence of hydrogen peroxide, chloride, and CPO and found that chloroform was the main product. Hoekstra et al. (*20*) demonstrated that the CPO-mediated reaction of chloride and hydrogen peroxide with humic acids resulted in the formation of chloroform as a main product and chloroacetic acids, chloroacetones, chloropyruvic acids, and chloromaleic/fumaric acids as minor compounds. In this study, CPO originated from the fungus *Caldariomyces fumago*.

CPO activity has been observed in several soil extracts (*15, 21*). Laturnus et al. (*15*) observed high chlorination activity in the organic layer and about 1000-fold lower chlorination activity in deeper soil layers (15−180 cm). These findings support our observation that at deeper soil layers (10−160 cm) in the Douglas forest no formation of chloroform from $Na^{37}Cl$ was observed. Extracellular peroxidase-like enzymes from fungi are probably responsible for the chlorination reaction in soil extracts. Their catalytic chlorination activity depends on the individual species and pH. In general, optimum chlorination yields occur for pH 3−5, which is the typical pH range of soils with a humic top layer.

Basidiomycetous fungi produce *de novo* specific chlorinated aromatic compounds (*22*). *De novo* production of chloroform (*11*) is possibly a minor side reaction mediated by the chlorinating enzymes of the basidiomycetes because the amounts of chlorinated anisyl metabolites are produced in 45−660-fold higher amounts. Since basidiomycetes does not show CPO activity, it is still unknown which enzyme(s) is (are) responsible for the specific chlorination reactions involved. The *de novo* biosynthesis of chlorinated metabolites and the mechanism involving the natural formation of reactive chlorine species outlined above seemingly are

TABLE 1. Isotope Fraction of ^{37}Cl in Chloroform and Percentage of Chloroform Formed from $Na^{37}Cl$ in Soil Air of the Douglas and Beech Forest (1994−1995)[a]

	forest											
	Douglas								Beech			
	B1[d]		C1[d]		F2[d]		F3[d]		B2[d]		B3[d]	
period	*f*[b]	*w*[c]	*f*[b]	*w*[c]	*f*[b]	*w*[c]	*f*[b]	*w*[c]	*f*[b]	*w*[c]	*f*[b]	*w*[c]
7/1−7/15	0.25	2										
7/15−7/29	0.25	2										
9/9−9/23	0.42	32										
9/23−10/8	0.43	31										
10/8−10/22	0.38	24	0.24	0	0.25	1	0.25	2				
10/22−11/6	0.37	22	0.27	4	0.26	1	0.26	3	0.26	4	0.27	6
11/6−11/19	0.37	22	0.28	7	0.26	3	0.28	5	0.26	3	0.27	6
11/19−12/18	0.35	18							0.26	4	0.26	4
12/18−1/14	0.34	16	0.28	8	0.26	4	0.28	9				
1/14−1/28	0.32	15							0.25	2	0.26	3
1/28−2/18	0.34	16			0.27	5	0.28	7				
4/8−4/27	0.33	14	0.28	6	0.26	3	0.28	6				

[a] The line indicates soil disturbance. [b] *f*, isotope fraction of ^{37}Cl in chloroform. [c] *w*, percentage of chloroform formed from $Na^{37}Cl$ (wt %). [d] Site code.

TABLE 2. Concentrations (ng/L) of Chloroform, 1,1,1-Trichloroethane, and Bromodichloromethane in Soil Air and pH at Several Depths (cm) of Site B1 in the Douglas Forest (1994−1995)

	period						
depth	9/23−10/8	10/8−10/22	12/18−1/14	1/14−1/28	1/28−2/18	4/8−4/27	pH
				$CHCl_3$			
−5	nm[a]	0.12	0.15	0.14	0.14	nm[a]	
10	6.0	10	14	2.0	4.0	8.3	3.6
20	18	15	14	18	18	22	4.0
40	21	26	21	28	18	30	4.2
80	22	21	21	27	19	27	4.2
120			36	36	nm[a]	24	4.2
160			18	28	16	26	4.2
				CCl_3-CH_3			
−5	nm[a]	nm[a]	1.4	1.3	0.98	nm[a]	
10	0.53	nm[a]	0.31	0.26	0.34	0.59	
20	0.75	nm[a]	0.59	0.78	0.95	0.92	
40	0.61	nm[a]	0.58	0.76	0.71	1.0	
80	0.51	nm[a]	0.62	0.76	0.92	0.98	
120			0.81	0.74	nm[a]	1.0	
160			0.55	0.79	0.83	0.83	
				$CHBrCl_2$			
−5	dl[a]	dl[a]	dl[a]	dl[a]	dl[a]	dl[a]	
10	0.033	0.035	nm[a]	nm[a]	0.03	0.03	
20	0.090	0.058	0.07	0.07	0.07	0.09	
40	0.10	0.10	0.10	0.11	nm[a]	0.11	
80	0.18	0.10	0.13	0.14	0.13	0.11	
120			0.31	0.23	nm[a]	0.16	
160			nm[a]	0.16	0.13	0.19	

[a] nm, not measured; dl, detection limit.

different pathways. However, until further proof is provided one should not rule out the possibility that some chlorinating enzymes may not be able to form reactive chlorine species but produce an activated enzyme-containing complex instead, which can accelerate specific chlorination reactions inside or outside the cell (*18*).

It is known from studies on the chlorination mechanism of organic matter in the drinking water disinfection process [eq 2] that chloroform is formed during chlorination of catecholic and resorcinolic structures in humic substances (*23*). In unpolluted soils, i.e., without any anthropogenic input of chlorinated products, Johansson et al. (*24*) demonstrated the existence of chlorinated phenolic and catecholic fragments in humic acids. The authors could not identify resorcinolic fragments, because these were oxidized during their analytical procedure. All these chlorinated aromatic fragments in humic acids contribute to the natural organochlorine content of soil which is in the range of 1−400 mg Cl/kg dry soil all over the world (*25*). In addition, the basidiomycetes can produce *de novo* specific chlorinated aromatic compounds, such as chlorinated anisyl, orcinolic, and hydroquinone compounds (*22*). This implies that there is a pool of potential chloroform precursors in soil.

Chlorination of humic substances by a low chlorine dose results in a large number of compounds containing a trichloroacetyl (TCA) group, and further chlorination causes the oxidation of these TCA-containing compounds (*26*). The formation of chloroform proceeds via hydrolysis of TCA-containing compounds, which preferably takes place at pH >7, after chlorination and ring opening of the aromatic structures (*23*). The CPO-mediated reaction probably produces a low dose of hypochlorous acid in soil which will react with soil organic matter to produce the TCA-type chloroform precursors. Hydrolysis of TCA to chloroform will be slow in our soil layers because of the low pH of 3−5. This process probably accounts for an almost constant concentration of chloroform in soil air in all seasons.

Concluding, our hypothesis is that chloroform is produced biogenically in the soil top layer together with TCA-type chloroform precursors. Fungi will play an important role in the production of chloroform and its precursors either by *de novo* biosynthesis or by the extracellular CPO-mediated mechanism. Chloroform which is produced in the soil top layer will be emitted to the atmosphere, while the "stable" TCA-type chloroform precursors will be transported by rainwater to deeper soil layers where they will be hydrolyzed to chloroform. The soil air concentrations suggest that the chloroform production in the top soil layer by *de novo* biosynthesis or by the extra-cellular CPO-mediated mechanism will be smaller than the chloroform production from TCA-type precursors in lower soil layers.

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