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Determination of volatile chlorinated hydrocarbons in plant leaves by gas chromatography–mass spectrometry

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Abstract

A method based on GC–MS was developed to determine concentrations of six C_1- and C_2-chlorinated hydrocarbons (trichloro- and tetrachloromethane, 1,2-dichloro- and 1,1,1-trichloroethane, trichloro- and tetrachloroethene) in plant foliage. The method basically consists of a solvent extraction of the plant leaves with pentane and analysis using a gas chromatograph–mass spectrometer in the selected-ion monitoring mode. Detection limits and calibration graphs were determined. The method was used to measure concentrations of chlorinated hydrocarbons in four different plant species. Only tetrachloroethene could be detected. The concentration was found to be dependent on the plant species. The bioconcentration factor of tetrachloroethene in one plant species was calculated and was found to be seven to twenty times lower than those of monocyclic aromatic hydrocarbons.

1. Introduction

C_1- and C_2-chlorinated hydrocarbons are important priority toxic pollutants that have been increasingly applied for the last decades as solvents for dry cleaning, as degreasing agents in metal industries or as fumigants. Some are also used in the manufacture of plastics and textiles and in the synthesis of other chemicals [1]. The global production of chlorocarbons used as solvents amounts to almost $3 \cdot 10^{12}$ kg ($3 \cdot 10^6$ tons) annually [2]. Because of their high volatility, it is estimated that about 70% escape to the atmosphere during application [3]. As C_1- and C_2-chlorinated hydrocarbons exhibit a high atmospheric stability, resulting in relatively long atmospheric lifetimes (from a few weeks to more than a hundred years) [4,5], they can be transported to areas far from the emission source. Average atmospheric concentrations in rural or forested areas vary from 0.3 to 1.2 $\mu g\ m^{-3}$, whereas in a city air concentrations are three- to six-fold higher [2,4,6]. Background levels of chlorinated hydrocarbons, measured on the island of Madeira, were twenty-fold lower than in the city and rarely exceeded 0.3 $\mu g\ m^{-3}$ [4]. Also in California, background concentrations in the range of 0.1 to 0.8 $\mu g\ m^{-3}$ and at least ten-fold higher levels in urban environments were measured [6].

Exposure to halocarbons can be injurious to human health: many of these compounds are toxic and exhibit mutagenic and/or carcinogenic properties [1,7] and some are involved in the

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degradation of the stratospheric ozone layer [7,8].

Once the halocarbons are released in the environment, they will partition between the different compartments of the ecosystem. An important property is their lipophilicity: they may accumulate in fat tissue of vertebrates and invertebrates. Also in lipophilic parts of plants accumulation can take place. Up to now, most studies about sorption of chemicals in plants consider semi-volatile or non-volatile chlorinated chemicals, such as PCB (polychlorinated biphenyls), DDT (dichlorodiphenyltrichloroethane), HCH (hexachlorohexane) or PCDD (polychlorodibenzodioxins) and PCDF (polychlorodibenzofurans) [9–13]. Only few studies reported the uptake of volatile chlorinated hydrocarbons in plant leaves. Frank and Frank [3] measured the concentration of tetrachloroethene in spruce needles [*Picea abies* (L.) Karst.], using solvent extraction and GC analysis with electron capture detection, whereas Plümacher and Renner [14] determined volatile chlorinated hydrocarbons and trichloroacetic acid in conifer needles by headspace gas chromatography.

The intent of this research was to develop a fast but sensitive method to determine concentrations of six C_1- and C_2-chlorinated hydrocarbons (trichloromethane, tetrachloromethane, 1,2-dichloroethane, 1,1,1-trichloroethane, trichloroethene and tetrachloroethene) in plant leaves. It is based on a solvent extraction of the plant material, followed by gas chromatographic–mass spectrometric analysis. Instrumental parameters, calibration graphs and detection limits were determined. The method was applied to compare concentrations of halocarbons in four different plant species, exposed to ambient air, in order to evaluate the role of vegetation as a sink for volatile chlorinated hydrocarbons.

2. Experimental

The method that was developed to determine concentrations of C_1- and C_2-chlorinated hydrocarbons in plant leaves basically consists of a solvent extraction of the plant leaves, followed by analysis of the extract in a gas chromatograph (GC), coupled with a mass selective detector (MSD).

2.1. Choice of extraction solvent

An appropriate solvent for the extraction of C_1- and C_2-chlorinated hydrocarbons from plant leaves should have the following properties: (i) it should be apolar; (i) it should elute from the column before the chlorinated compounds; (iii) it must not contain any traces of the compounds of interest.

Both hexane and pentane were tested as extraction solvents by injecting a standard solution of the chlorinated compounds in each solvent in the GC–MSD system. Using hexane (Aldrich, 95 + %, HPLC grade), only tetrachloromethane, trichloroethene and tetrachloroethene could be completely separated and integrated. The other halocarbons eluted too close to hexane. With pentane (Aldrich, 99 + %, HPLC grade) all chlorinated hydrocarbons could be detected. An important disadvantage of pentane however, is that due to its high volatility (boiling point 35°C), evaporation can take place during manipulations of sample preparation. This problem is obviated by the use of an internal standard.

2.2. Sample collection and preparation

From four plant species [*Pseudotsuga menziesii* (Mirb.) Franco, *Chamaecyparis lawsoniana* (Murr.) Parl., *Cotoneaster dammeri* Schn. 'Skogholm' and *Abies grandis* (D. Don) Lindl.], leaves of three different plants were sampled in January 1994. All plants were growing on the central reservation of a main street in the city of Ghent within a distance of 800 m from each other, so they can be assumed to be exposed to the same global level of air pollution.

Leaves were cut from the twigs using scissors and collected in glass vials of 4 ml provided with screw caps. In each vial, approximately 1 g of fresh leaves was collected. Sampling of all plants was carried out within two hours. Immediately after sampling, extraction solvent was added to

the leaves (1 ml of solvent for 1 g of fresh leaves). The extraction solvent used was pentane (Aldrich, 99 + %, HPLC grade), to which an internal standard was added (concentration 5 pl μl^{-1}). As the internal standard should be an apolar compound that is absolutely absent in ambient air, perdeutero-octane was chosen.

After 6 h of extraction, which was found to be the optimum extraction time to extract monocyclic aromatic hydrocarbons from plant leaves [15], extracts were filtered (Millex HV 0.45 μm) to remove dust or soot particles. Aliquots of 1 μl of the filtrate were injected into the GC–MSD system.

2.3. Instrumental parameters and analysis

A Hewlett-Packard (HP) Model 5890 gas chromatograph equipped with a HP Model 5970 A quadrupole mass spectrometer and a Model 200 HP computer system was used to analyse the plant extracts. The GC was provided with a 50 m × 0.258 mm ID fused-silica capillary column coated with a 0.25 μm thick layer of a 5% phenyl–95% methyl polysiloxane stationary phase (DB-5; J&W Scientific). Splitless injection was used. The carrier gas was helium. The temperature program was as follows: initial temperature: 30°C; initial time: 7 min; rate: 2°C min^{-1} from 30°C to 48°C; rate: 16°C min^{-1} from 48°C to a final temperature of 240°C; final time: 5 min. In this way, the total analysis time was confined to 33 min. The solvent delay was set at 3.90 min. At 16 min, when the rate of temperature increase was changed, all compounds of interest were already eluted from the column, so data acquisition of the MSD was switched off. In this way, terpenes which can be present in large amounts in the plant extracts and which exhibit retention times of 16 min or more are not recorded and are removed quickly from the column. The injector temperature was set at 250°C and the GC–MS interface temperature was 260°C.

The mass selective detector was programmed in the Selected Ion Monitoring (SIM) mode in order to work selectively and sensitively. According to their retention times, the chlorinated hydrocarbons were gathered in four groups. For the mass selective detection of each compound, the masses corresponding to the three or four largest peaks in the mass spectrum were chosen. All selected masses for data acquisition and the corresponding time interval for each group of compounds are given in Table 1.

3. Results and discussion

Using the instrumental parameters and SIM program described above, detection limits and calibration graphs of the six halocarbons were determined. The method was then tested with leaves of four plant species.

Table 1
Parameters for data acquisition in Selected Ion Monitoring (SIM) mode

Group	Time interval for sampling (min)	*m/z*	Compound to be detected
1	3.90–4.75	47, 83, 85	Trichloromethane
2	4.75–6.50	61, 97, 99	1,1,1-Trichloroethane
		49, 62, 64	1,2-Dichloromethane
		117, 119, 121	Tetrachloromethane
3	6.50–10.00	95, 97, 130, 132	Trichloroethene
4	10.00–13.30	66, 82, 98	Perdeutero-octane
5	13.30–16.00	129, 131, 164, 166	Tetrachloroethene

3.1. Detection limits

The detection limit for each volatile chlorinated compound, which is defined as the amount of compound that gives a signal-to-noise ratio of three, was determined by diluting a stock solution of 1 pl μl^{-1} for the six halocarbons to a concentration of 0.01 pl μl^{-1}. Exactly 1 μl was injected in the GC–MSD. From the signal-to-noise ratio obtained for 0.01 pl, the amount of compound (in pg) corresponding to a signal-to-noise ratio of three could be calculated. The detection limits in this way obtained (mean of five injections) were as follows: trichloromethane: 5.0 pg; 1,1,1-trichloroethane: 8.2 pg; 1,2-dichloroethane: 15.6 pg; tetrachloromethane: 10.9 pg; trichloroethene: 7.8 pg; tetrachloroethene: 8.6 pg.

Relative standard deviations varied from 9.3 to 15.2%.

3.2. Calibration graphs

Calibration graphs were obtained by injecting six standard solutions containing a mixture of the chlorinated hydrocarbons in concentrations of 1, 0.75, 0.5, 0.25, 0.1 and 0.05 pl μl^{-1} respectively and a constant concentration of the internal standard perdeutero-octane of 5 pl μl^{-1}. In this way, it could be checked whether concentrations approaching the detection limit still gave a linear response in the MSD. By plotting the ratio of the peak area of the chlorinated compound to the peak area of the internal standard against the corresponding concentration, six linear calibration graphs were obtained with a correlation coefficient of minimum 0.998 (Fig. 1).

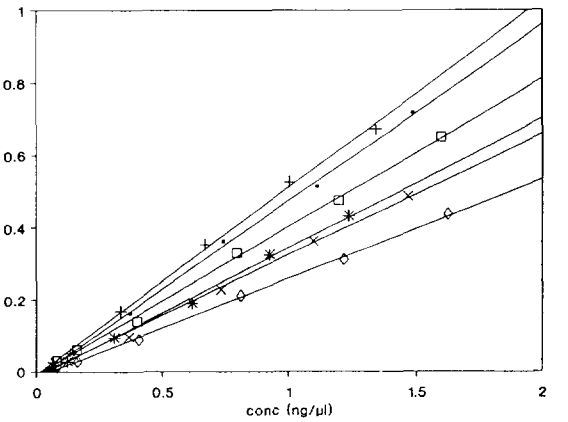

Fig. 1. Calibration graphs of trichloromethane (·), tetrachloromethane (□), 1,2-dichloroethane (*), 1,1,1-trichloroethane (+), trichloroethene (×) and tetrachloroethene (◇).

3.3. Analysis of plant extracts

With the method described, leaf extracts from the species *Pseudotsuga menziesii*, *Chamaecyparis lawsoniana*, *Cotoneaster dammeri* and *Abies grandis* were analysed. Chromatograms of extracts of *Pseudotsuga* and *Chamaecyparis* needles are shown in Figs. 2 and 3, respectively. In these chromatograms, the largest peak with a retention time of resp. 12.547 and 12.567 min corresponds to the internal standard perdeutero-octane, while the peak eluting at resp. 13.768 and 13.792 min represents tetrachloroethene. The peak with retention time of resp. 13.327 and 13.325 min is hexanal, which was identified using the full-scan mode. After carefully examining retention times and mass spectra of the compounds in the chromatograms and comparing it with those in the standard solution, it could be concluded that all other peaks in Figs. 2 and 3 are none of the chlorinated hydrocarbons. This was also the case for the extracts of the other plant species: in none of them trichloro- and tetrachloromethane, 1,2-dichloro- and 1,1,1-trichloroethane and trichloroethene could be detected. Apparently, these compounds are too volatile (with high air/plant partition coefficients) and/or are present in too low concentrations in ambient air to be taken up in detectable concentrations in plant leaves.

In Table 2, the concentrations of tetrachloroethene in the four plant species examined are shown. In *Pseudotsuga menziesii* highest concentrations were measured (two and ten times higher than in *Chamaecyparis* and *Abies*, respectively), while in leaves of *Cotoneaster dammeri* no tetrachloroethene was present at all. Apparently, concentrations are highly dependent on the plant species. Plümacher and Renner [14] also observed differences in concentrations of

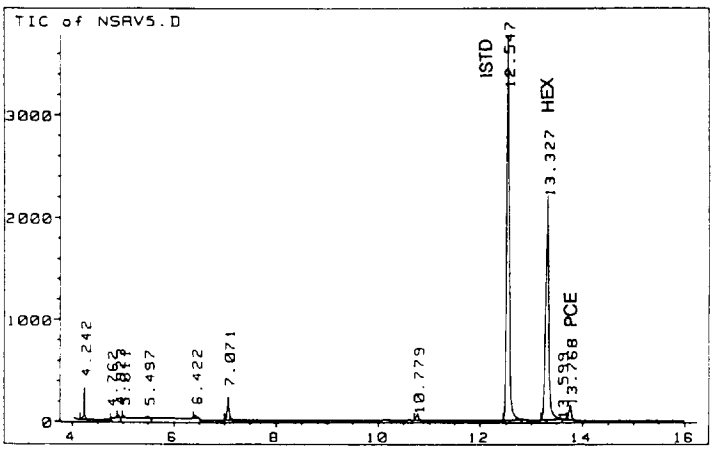

Fig. 2. Chromatogram of a *Pseudotsuga menziesii* extract with in abcis retention time (min) and in ordinate abundance (arbitrary units) with ISTD: internal standard; HEX: hexanal; PCE: tetrachloroethene.

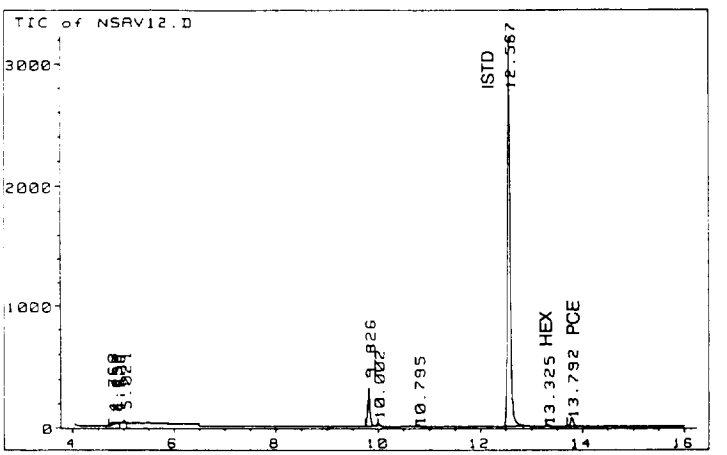

Fig. 3. Chromatogram of a *Chamaecyparis lawsoniana* extract with in abcis retention time (min) and in ordinate abundance (arbitrary units) with ISTD: internal standard; HEX: hexanal; PCE: tetrachloroethene.

Table 2
Concentrations of tetrachloroethene (ng g^{-1} leaf dry weight) in leaves of *Pseudotsuga menziesii*, *Chamaecyparis lawsoniana*, *Abies grandis* and *Cotoneaster dammeri* with mean, standard deviation (*s*) and coefficient of variation (CV) in three specimens

	Plant number			Mean	*s*	CV
	1	2	3			
Pseudotsuga menziesii	230	257	179	222	40	18%
Chamaecyparis lawsoniana	92	113	116	107	13	12%
Abies grandis	32	22	29	28	5	18%
Cotoneaster dammeri	–	–	–	–	–	–

volatile chlorinated hydrocarbons between *Abies alba* Mill., *Picea abies* (L.) Karst. and *Pinus sylvestris* L.

To compare previously reported concentrations of chlorinated hydrocarbons in plant leaves [3,14] with the present data, a leaf density of 0.89 g cm^{-3} and a water content of 0.7 g water g^{-1} wet leaf are assumed [16], in order to express all concentrations in ng g^{-1} dry weight (d.w.). Plümacher and Renner [14] determined up to 90 ng g^{-1} d.w. tetrachloroethene in *Abies alba* and 15 ng g^{-1} d.w. in *Picea abies*, both species growing at a forest site. They also found no trichloroethene in the three species examined and only low concentrations (maximum 5 ng g^{-1} d.w.) of 1,1,1-trichloroethane and tetrachloromethane. Frank and Frank [3], on the other hand, measured 7.5 ng g^{-1} d.w. tetrachloroethene in *Picea abies* needles in a city. In the present study, concentrations of tetrachloroethene vary from 28 ng g^{-1} d.w. in *Abies grandis* to 222 ng g^{-1} d.w. in *Pseudotsuga menziesii*, a species which is known to sorb up to 100 times higher concentrations of toluene, ethylbenzene and xylenes than other conifer species such as *Pinus nigra* Arnold. [17]. Taking into account that city air concentrations are three to six times higher than forest air levels [4], the concentrations found in this study are comparable with those of Frank and Frank [3], who also used a similar determination method (solvent extraction with hexane and GC analysis with ECD detection).

The affinity of plant leaves to sorb chemical compounds can be expressed by a bioconcentration factor (BCF_V), which is the ratio of the concentration in the leaves (in $\mu g\ m^{-3}$ of wet leaf) to the concentration in the air (in $\mu g\ m^{-3}$) and which can be calculated according to a fugacity-based model developed by Paterson et al. [16]. For one plant species, *Pseudotsuga menziesii*, data are available to allow comparison of the bioconcentration factor of tetrachloroethene with those of monocyclic aromatic hydrocarbons (MAH) (toluene, ethylbenzene and xylenes). Needle concentrations of monocyclic aromatic hydrocarbons in *Pseudotsuga menziesii* were measured in the same trees and during the same time of the year (January), following a method described by Keymeulen et al. [15]. Mean values in the three *Pseudotsuga* trees were 16.3 $\mu g\ g^{-1}$ d.w. toluene, 10.2 $\mu g\ g^{-1}$ d.w. ethylbenzene, 33.9 $\mu g\ g^{-1}$ d.w. *m,p*-xylene and 15.3 $\mu g\ g^{-1}$ d.w. *o*-xylene. Mean air concentrations of MAH in Ghent at the sampling site were 35.5 $\mu g\ m^{-3}$ toluene, 8.0 $\mu g\ m^{-3}$ ethylbenzene, 25.1 $\mu g\ m^{-3}$ *m,p*-xylene and 10.1 $\mu g\ m^{-3}$ *o*-xylene [17]. Since for the air concentrations of tetrachloroethene no data from Ghent or other cities in Belgium are available, average levels from cities in Germany have to be used. In Tübingen, a moderately industrialized city with 80 000 inhabitants, the median value of 30–40 measurements was 3.31 $\mu g\ m^{-3}$ [4]. This concentration is in agreement with the measurements of Bruckmann et al. [18] in a densely populated urban area along a street with dense traffic in the city of Hamburg (3.9 $\mu g\ m^{-3}$). It reflects a global urban air level when no emission sources (dry cleaning or metal degreasing factories) in the direct neighbourhood are present, as it was the case at the sampling site of the *Pseudotsuga menziesii* trees in Ghent. As Ghent is a moderately industrialized city with 200 000 inhabitants, an average tetrachloroethene concentration at the sampling site of 3.5 $\mu g\ m^{-3}$ is assumed.

With the data mentioned, the following bioconcentration factors in *Pseudotsuga menziesii* needles are obtained: toluene: $1.2 \cdot 10^5$; ethylbenzene: $3.4 \cdot 10^5$; *m,p*-xylene: $3.6 \cdot 10^5$; *o*-xylene: $4.0 \cdot 10^5$ and tetrachloroethene: $1.7 \cdot 10^4$. Apparently, the bioconcentration factor of tetrachloroethene is about seven times lower than that of toluene and about twenty times lower than the BCF_Vs of the C_2-benzenes, which means that the *Pseudotsuga menziesii* needles have a lower affinity for tetrachloroethene than for the MAH.

4. Conclusions

With the proposed method, concentrations of volatile chlorinated hydrocarbons can be measured in plant leaves. In the four plant species

tested, only tetrachloroethene could be detected. Apparently, the other compounds are too volatile and/or are present in concentrations in ambient air too low to be taken up by plant foliage. Sorption in plant leaves also seems to be highly dependent on the plant species.

The bioconcentration factor (BCF_V) of tetrachloroethene in *Pseudotsuga menziesii* needles was compared to the BCF_Vs of monocyclic aromatic hydrocarbons (MAH) in this species and it was found to be seven to twenty times lower than BCF_Vs of MAH.

It can be concluded that from the six volatile chlorinated hydrocarbons considered, vegetation can only be a sink for tetrachloroethene, but to a less extent than for the monocyclic aromatic hydrocarbons.

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