The distribution of chlorinated solvents in the air and foliage of three British forests

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Declaration

Except where specific reference is made to other sources, the work presented in this thesis is the original work of the author. It has not been submitted, in part or in whole, for any other degree. Certain of the results have been published elsewhere.

The thesis describes the results of research carried out in the department of Chemistry, University of Edinburgh, under the supervision of Dr. J.G. Farmer and Dr. J.N. Cape (Institute of Terrestrial Ecology, Bush Estate, Edinburgh) since 1st October 1991, the date of my admission as a research student.

Ruth Brown
29th August 1995

For Mum and Dad

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Abstract

Volatile C_1 and C_2 chlorinated hydrocarbons and their oxidative degradation products are possible contributors to forest decline in central Europe and north America. These species originate from both natural and anthropogenic sources, the latter including use as solvents and degreasers within the chemical, dry-cleaning and nuclear industries.

Concentrations have been reported in European forest air, soil and foliage but no measurements have been made in the UK. This study presents the temporal and spatial distribution of 1,1,1-trichloroethane, tetrachloromethane, trichloroethene and tetrachloroethene in air and foliage of three northern UK forests over a 15 month period. Potential sources were identified as petrochemical industry at Grangemouth (Central Region, Scotland) and the nuclear reprocessing complex at BNFplc. Sellafield (Cumbria, England). A 'clean' site (Grampian, Scotland) was included for comparison. The tree species monitored was *Pinus sylvestris* (L).

Air measurements were made using novel passive tube samplers containing an extract of *Pinus sylvestris* (L.) wax exposed to the atmosphere for approximately one month. Analysis of the tubes and composite needle samples used solvent extraction and quantitative capillary gas chromatography with electron capture detection. Complementary GC/MSMS was used qualitatively.

The needle concentrations found were similar to those of central Europe, and were log-normally distributed. Geometric mean concentrations (ng/g dry weight) were $C_2H_3Cl_3$ 20; CCl_4 5; C_2HCl_3 130; C_2Cl_4 30. There was no statistically significant difference at the 95% level between median concentrations at the three sites, or between the 1992 and 1993 needle year classes. The geometric standard deviation of 2-3 was typical of other gaseous pollutants, and the vertical profile through the canopy at Devilla (near Grangemouth) suggested an atmospheric source. Comparison of temporal variation with wind direction was consistent with the possibility that the petrochemical complex was a source, but there was no evidence of a significant source at Sellafield. There was no correlation between high needle concentrations and any observed decline in tree health.

The concentrations measured in *Pinus sylvestris* (L.) needles were proportional to amounts in co-sampled air diffusion tubes. Laboratory determinations of the partition coefficient ($kg.m^{-3}/kg.m^{-3}$) for each chlorinated hydrocarbon (at concentrations some 10^6 larger than in air) between wax and air, and whole needles and air, were similar to those previously reported for spruce: wax 80 (C_2HCl_3) - 700 (C_2Cl_4); needles 13 ($C_2H_3Cl_3$) - 400 (C_2Cl_4).

The partitioning of chlorinated solvents between the wax used in passive samplers and whole needles, exposed to the same air concentrations, was not significantly different for field samples exposed to ambient air, and samples exposed to 10^6 larger concentrations in the laboratory. The passive diffusion samplers can therefore be used as well-controlled surrogates for measuring concentrations of chlorinated solvents in pine needles in the field. The similarity in the partitioning between wax and whole needles in ambient air and in the laboratory, and the lack of accumulation with time in the field, suggest that only a small proportion of sorbed solvent is held irreversibly in pine needles. However, the unrealistically large air concentrations predicted from field exposed wax in the passive diffusion tubes and from whole needles, using the partition coefficients determined in the laboratory, suggest a marked non-linearity in the partition coefficients at ambient concentrations for both wax and whole needles.

General contents

Contents

Chapter 1 Introduction

Chapter 2 Experimental : Sampling methods and preliminary investigations

Chapter 3 Experimental : Gas chromatographic analysis

Chapter 4 Partition ratio studies

Chapter 5 Statistical methods in the analysis of gas chromatography data

Chapter 6 Results and discussion : Chlorinated solvents in pine needles

Chapter 7 Results and discussion : Passive air samplers

Chapter 8 Conclusions and discussion of relevance to forest decline

Appendices

List of Figures

Page

Chapter 1 Introduction

Chapter 2 Experimental : Sampling methods and preliminary investigations

Chapter 3 Experimental : Gas chromatographic analysis

Chapter 4 Partition ratio studies

Chapter 5 Statistical methods in the analysis of gas chromatography data

Chapter 6 Results and discussion : Chlorinated solvents in pine needles

Chapter 7 Results and discussion : Passive air samplers

List of Figures : Appendices

Appendix 4 : The theory of gas chromatography

Appendix 6 Determination of the conversion ratio from needle dry weight to surface area for a typical batch of needles

List of Tables

Page

Chapter 1 Introduction

Chapter 2 Experimental : Sampling methods and preliminary investigation

Chapter 3 Experimental : Gas chromatographic analysis

Chapter 4 Partition ratio studies

Chapter 5 Statistical methods in the analysis of gas chromatography data

Chapter 6 Results and discussion : Chlorinated solvents in pine needles

Chapter 7 Results and discussion : Passive air samplers

List of Tables : Appendices

Appendix 4 : The theory of gas chromatography

Appendix 6 : Determination of the conversion ratio from needle dry weight to surface area for a typical batch of needles

Appendix 8 : Example calculation for the BCF

Chapter 1

Introduction

"“Magic!” they exclaimed, swigging their drams in a most unboffinly manner: “But magic is merely undiscovered science and we'd like to take some home with us for further investigation”"

as seen in New Scientist

1 INTRODUCTION

1.1 Overview

In recent years air pollution has become more extensive, with the general trend being towards more effective dispersion from major sources. As a consequence, smaller concentrations of pollutant gases are seen close to sources, while concentrations in remote areas are larger than might be expected (Fowler and Cape, 1982; Hellqvist *et al.*, 1992). The widespread nature of many atmospheric pollutants raises questions about their real and potential impact on the environment (Osswald and Elstner, 1987).

The properties of C_1 and C_2 chlorinated hydrocarbons have led to widespread use in an increasing number of technical applications. The sources of these compounds are predominantly anthropogenic although natural sources are known (Gribble, 1994; Hoekstra and de Leer, 1995), and production has risen rapidly over the last few decades with consumption being almost entirely equal to the losses by evaporation into the environment (Frank and Frank, 1990). The annual global production of the C_2 chlorinated hydrocarbons is presently over two million tonnes (Midgeley, 1992).

Concern about the depletion of the ozone layer has led to severe limits being placed on the production and use of some C_1/C_2 chlorinated hydrocarbons under the dictates of the Montreal Protocol (Tierney, 1995). However, their relative chemical stability, which confers upon them their useful properties, also leads to long atmospheric lifetimes and their presence in the environment is assured for the foreseeable future. For the same reason, the differences in air concentrations between urban/industrial and rural/remote regions are less marked than for the majority of other pollutants (Singh, 1979).

Volatile C_1/C_2 chlorinated hydrocarbons and their oxidative degradation products have been investigated as possible contributors to the type of forest decline observed mainly in central Europe and North America (Frank *et al.*, 1994; Plümacher and Renner, 1993).

The bleaching of tree foliage (chlorosis) and premature needle drop was initially observed during the 1970's in Silver fir (*Picea alba*) and Norway spruce (*Picea abies* (L.) Karst) growing at high altitudes in the Bavarian and Black forests. Several other coniferous species, including Scots pine (*Pinus sylvestris* (L.)), are now affected and the damage has spread to deciduous trees and forests of younger trees at lower altitudes (Schutt, 1983). The damage appears to be greater in exposed, high altitude forests, and in needles on the upper side of branches, leading to attempts to correlate observed decline with light levels (Osswald and Elstner, 1987). In the UK, this type of visible tree damage is not as severe as that seen in central Europe and north America, and levels of discoloured needles remain very low (Innes, 1991).

The extent, causes and diagnosis of damage to forests have widely been considered in terms of air pollutant distribution (Cape, 1986; Frank and Frank, 1986; Garrec *et al.*, 1988; Percy *et al.*, 1993 (*in press*); Weigel *et al.*, 1989). The forests most severely affected by this type of decline are generally in regions classed as 'clean air' with respect to SO_2 and NO_x (Osswald and Elstner, 1987). The relationship between this type of damage and atmospheric photooxidants such as ozone has also been studied (Mather,1994; Osswald and Elstner, 1987).

Recent research stresses that normal physiological and ecological reasons for the effects must be considered in parallel with possible pollutant inferences (Bermedinger-Stabentheiner, 1993; Hellqvist *et al.*, 1992). Exposure to atmospheric pollutants appears to alter the sensitivity of trees to some natural agents such as wind, drought, snow, dew, frost and pests (van Gardingen *et al.*, 1991; DoE, 1993) and accelerates natural foliage ageing processes (Bermadinger-Stabentheiner, 1993; Günthardt-Goerg, 1986). However, in the field, natural and artificial influences are difficult to separate (Günthardt-Goerg, 1994 *in press*) and assignment of air pollutants to a specific cause of damage, such as structural wax degradation, must be made with care, particularly when exposure to multiple chemicals is involved (Vong and Guttorp, 1991). A synergistic hypothesis for forest decline incorporating both abiotic and biotic influences is suggested. For example, injured epicuticular wax structures favour needle infestation by parasites, and a structurally or chemically altered surface may influence the uptake of pollutants (Bermadinger-Stabeutheiner, 1993).

The observed overall decline in tree health in Britain during recent years is widely accepted to be primarily caused by climatic and other natural stresses, with air pollution acting as an additional, and as yet unquantified, stress (DoE, 1993; Innes, 1991; Mather, 1994). Some signs of improvement in tree condition for some species, including Scots pine, have been reported by the Forestry Authority (Redfern *et al.*, 1995).

No links can be established between pollution and forest condition until more information is available on parameters such as insect pest status, and the likely effects of mechanical damage by wind, snow and storms on forest condition can be accounted for in models (Mather, 1994). The DoE report of 1993 states that 'acute air pollution damage to trees is rare in Britain, but in some areas of the UK it is likely that the pollution climate contributes to tree damage'. Particular air pollutants of interest in the report were ozone, and SO_2 and NO_x, primarily in the context of acid mists. A fraction of the crown dieback in beech (*Fagus sylvatica* (L.)) and change in flowering of Scots pine can be correlated with atmospheric ozone concentration, as can crown density changes in Norway spruce (Mather, 1994).

The epicuticular wax layer on plant leaves has been the focus of much interest with respect to air pollutants, as it is interposed between the plant and its environment, and is one of the first targets of all influences that reach the surface from the outside (Baker, 1982; Bermadinger-Stabentheiner, 1993). This wax layer is important in gas exchange processes (Kerstiens, 1993 *in press*), alterations will influence the transportation of gases and water, and photosynthetic mechanisms.

Measurable concentrations of C_1/C_2 chlorinated hydrocarbons, and their degradation products, have been detected in forest foliage, air, soil and earthworms (Black and Susser, 1992; Diezel *et al.*, 1988; Frank and Frank, 1989; Juuti *et al.*, 1993; Plümacher and Renner, 1993 *in press*). The lipophilic nature of these compounds, as indicated by their high Ostwald solubility coefficients and high values of Henry's law constant which reflect low solubility in water, suggests that they may be enriched in the epicuticular wax and cellular membranes (Frank and Frank, 1986). At a given environmental concentration, equilibrium concentrations of organic chemicals in plants increase with increasing lipophilicity and decreasing vapour pressure (Schreiber and Schönherr, 1993).

Possible mechanisms by which these compounds may exert influence include destruction of major photosynthetic pigments in the presence of UV radiation (Frank and Frank, 1985) or through herbicidal effects of oxidative degradation products such as chloroacetates (Fuchs and Bächmann, 1987; Norokorpi and Frank, 1993 *in press*).

When assessing the concentrations of airborne chemicals to which forests are exposed, two aspects of their distribution must be considered (Fowler and Cape, 1982) :

(1) spatial distribution to assess the scale of potential effects (Cape *et al.*, 1988);

(2) frequency of occurrence to allow controlled environment studies of the 'effect' of 'typical' mixtures of gases on tree performance (Barns *et al.*, 1989).

The lack of long-term foliage and air monitoring data for forest sites - with regard to number of sites, variation in location and number of sampled trees, branches and needles- causes problems when attempting to quantify the effects of air pollutants on forest health (Fowler and Cape, 1982; Günthardt-Goerg, 1994 *in press*). This makes it particularly difficult to separate climatic and man-made influences.

Various plants and lichens have been used as bioindicators to monitor air quality (Klump *et al.*, 1994; Showman, 1988). Previous authors have indicated that pine and spruce needles are well suited to reflect concentrations of lipophilic chlorinated hydrocarbons such as PCBs and HCHs in the ambient air (Gaggi *et al.*, 1985; Reischl *et al.*, 1988). It therefore may be possible to use needle concentrations of the more volatile C_1/C_2 chlorinated hydrocarbons as a biomonitoring technique which can reflect forest stress levels, whether or not much damage is observed. As previously mentioned, assigning air pollutants to a specific cause of damage must be made with extreme care if multiple chemical stresses are suspected to be operating in conjunction with biotic influences (Showman, 1988).

There is certainly a place for further development of reliable methods of early diagnosis of air pollution effects, possibly using parameters such as amounts of needle surface wax, contact angles (wettability index) and foliar pigment ratio changes, e.g. chlorophyll/carotenoid, (Hellqvist *et al.*, 1992; Turunen and Huttunen, 1990). Such diagnostic techniques must be reasonably insensitive to natural differences between site and also sampling date, weather and time of day (Cape *et al.*, 1988).

1.2 Detailed introduction

This section provides the general and project-specific background to some aspects of C_1/C_2 chlorinated hydrocarbon distribution and uptake in UK forests.

The halogenated hydrocarbons studied in this project are selected from the group of volatile C_1/C_2 chlorinated hydrocarbons. Selection of these particular compounds was made on the basis of their likely environmental, industrial and political significance (Frank 1991; Gribble, 1994; Hoekstra and de Leer, 1995; Midgeley, 1989). The chosen subset of this group consists of $CHCl_3$ (trichloromethane), $C_2H_3Cl_3$ (1,1,1-trichloroethane), CCl_4 (tetrachloromethane), C_2HCl_3 (trichloroethene) and C_2Cl_4 (tetrachloroethene). Iodomethane (CH_3I) is also included in this section because of its use as an internal standard for the analytical method (Chapters 2 and 3).

The spatial distribution of each target compound at a rural UK forest site was compared with that of forest sites near two possible sources. The potential sources investigated were a nuclear installation (British Nuclear Fuels Sellafield, West Cumbria, England) and petrochemical industry at Grangemouth, Central Region, Scotland (Section 1.3).

1.2.1 Production, consumption and properties of chlorinated solvents

Production of chlorinated C_1/C_2 hydrocarbons

The natural production of different types of chlorinated compounds, ranging from CFCs and dioxins to simple alkanes and related species, is presently an area of great interest (Asplund and Grimvall, 1991; Gribble, 1994). Biogenic sources of substances such as chlorinated ethanes have only recently been acknowledged (Gribble, 1994; Mross and Konietako, 1991), and that such sources exist for others such as dioxins is still a matter for debate (Hoekstra and de Leer, 1995; Svenson *et al.* 1990).

Production of CCl_4 and all the C_2 chlorinated compounds investigated in this study is primarily via anthropogenic rather than natural processes. However, as **Table 1.2.1.1** shows, various biogenic sources are recognised (Gribble, 1994; Hoekstra and de Leer, 1995; Mross and Konietzko, 1991). The situation is different for $CHCl_3$ and CH_3I, as there is documented evidence for significant, and in some cases massive, production from natural sources (Hoekstra and de Leer, 1995; Isidorov, 1990).

Table 1.2.1.1 Biogenic sources of some halogenated hydrocarbons

Compound	**Sources**	**References**
CH_3I	Oceans, marine algae, volcanoes	Gschwend *et al.*, 1985 Harper, 1995; Tsetsi *et al.*, 1989
$CHCl_3$	Marine algae, lemon, white cedar, orange, moss, barley, drill wells, volcanoes, soil	Frank *et al.*, 1989 Hoekstra and de Leer, 1995 Isidorov, 1990
CCl_4	Marine algae, oceans, volcanoes, drill wells, soil	Frank *et al.*, 1989; Harper, 1995; Isidorov, 1990
CH_3CCl_3	Oceans	Grimvall and de Leer, 1995
C_2HCl_3	Oceans Volcanoes	Stoiber *et al.*, 1971 Grimvall and de Leer, 1995
C_2Cl_4	Oceans Volcanoes	Isidorov, 1990 Grimvall and de Leer, 1995

Natural production of tribromomethane is thought to be greater than that of trichloromethane (Harper, 1995). The major biogenic source of both is the marine environment, where both the ocean and algae may secrete such substances as 'anti-predator' chemicals (Sturges *et al.*, 1992). Various mechanisms have also been proposed for the production of $CHCl_3$ and CCl_4 in soils from substrates such as trichloroacetyl chloride (CH_3COCl) or chloride ions (Frank *et al.*, 1989).

The oceans are also the major source of chloromethane (CH_3Cl), which is the most abundant volatile halocarbon in the atmosphere and accounts for 20% of the chlorine in the stratosphere (Harper, 1995). The main source of CH_3I is also the ocean, and atmospheric levels are markedly higher (10-20 pptv) in oceanic regions of high algal productivity (Harper, 1995; Nightingale, 1991).

As demand has risen, anthropogenic production of all the studied compounds has increased over the last three decades (Midgeley, 1989), and has only recently been limited for some under the Montreal Protocol (Section 1.2.2) (Tierney, 1995). Global production of chlorinated solvents is around 3 million tonnes annually, with production of C_2Cl_4 in Britain currently at around 45 ktonnes per annum (Brooke *et al.*, 1991; Frank *et al.*, 1991). **Table 1.2.1.2** provides estimated global production, emissions and background levels of the compounds of interest.

Consumption and emissions of chlorinated C_1/C_2 hydrocarbons

Substances such as $C_2H_3Cl_3$, CCl_4, C_2HCl_3 and C_2Cl_4 were originally introduced for a broad spectrum of technical uses. Applications include use as solvents and degreasing fluids in metal and textile industries (Brooke *et al.*, 1991), and as dry cleaning solvents (Gulyas and Hemmerling, 1990).

Within the nuclear industry, use is mainly limited to within the laboratory (Hall, 1995), although some sites use trichloromethane, 1,1,1-trichloroethane and tetrachloromethane as coolants and cleaning agents, and in chemical processing (Sturges and Taylor, 1990). Loss from these sites is predominantly through evaporation and release to water (McNab and Narasimhan, 1994). Some waste is stored or incinerated.

Table 1.2.1.2 Estimated global production and background levels of some halogenated hydrocarbons.

Compound	Production (kt/year) Industrial	Production (kt/year) Natural	Emissions (kt/year)	Background levels (pptv) atmosphere	Background levels (pptv) forest air	Atmospheric lifetime (years)	References
CH_3I	-	Natural	300-3000	0.1-2.5		0.02	Harper, 1995; Tsetsi *et al.*, 1989
$CHCl_3$	250	+ Natural	20	20-30	10-90	0.3-0.6	Bächman and Polzer, 1989; Frank and Frank, 1990; Harper, 1995; Kelly *et al.*, 1994
CH_3CCl_3	600	+ Possibly some	520-720	130-160	10-190	5.4±0.6	Frank, 1991; Frank and Frank, 1990; Harper, 1995; Hisham and Grosjean, 1991; Plümacher and Renner, 1991; Smidt, 1992
CCl_4	1000	+ Some	30-110	95-145	15-120	42	Bächman and Polzer, 1989; Bloemen and Burn, 1993; Frank and Frank, 1990; Harper, 1995; Kelly *et al.* 1994; Plümacher and Renner, 1991;
C_2HCl_3	600	+ Some	500-600	10	30-340	0.02	Frank, 1991; Kelly *et al.*, 1994; Plümacher and Renner, 1991
C_2Cl_4	1100	+ Some	400-900	20-40	120-270	0.4	Bächman and Polzer, 1989; Frank, 1991; Kelly *et al.*, 1994; Plümacher and Renner, 1991

The amounts used within the petrochemical industry are larger and the applications more diverse, including initial and intermediate products for chemical synthesis processes and as solvents for cleaning and extraction procedures (Mross and Konietzko, 1991). Loss is again predominantly through release to the air and water compartments (Brooke *et al.*, 1991; McNab and Narasimhan, 1994).

Emissions in the UK range from 22 ktonnes ($C_2H_3Cl_3$) to <1 ktonne (CCl_4) per annum (Passant, 1993). In Britain, 95% of the 45000 tonnes of C_2Cl_4 produced annually is released into the environment via dry cleaning establishments. Estimation of geographical distribution of emissions is important, particularly for the shorter-lived compounds. The USA and Japan emit the largest amounts of 1,1,1-trichloroethane, followed by the European Union (Midgeley *et al.*, 1995). Concentrations of these compounds found in the air and foliage of forests are listed in Chapter 6.

Properties and reactions of chlorinated C_1/C_2 hydrocarbons

The properties of these substances which make them particularly suitable for the types of applications listed include low acute toxicity, non-inflammability and hydrolytic stability (Brooke *et al.*, 1991).

The ozone depletion potential of a halogenated compound is based on the degree of saturation (inertness) and H content, and is therefore reflected in the atmospheric lifetime (see **Table 1.2.1.2**). CCl_4 is the main O_3 depleting chemical of the chosen group as it is saturated and no H atoms are present to react with hydroxyl radicals in the troposphere (atmospheric lifetime is 42 years). It therefore reaches the stratosphere where atomic chlorine is formed which reacts with and therefore destroys ozone (Thompson, 1991). This chemical stability in the troposphere leads to persistence and ubiquitous distribution arising from transportation over long distances (Section 1.2.3). Unsaturated species such as 1,1,1-trichloroethane have a shorter atmospheric lifetime (5.4 years) but distribution is still widespread. The degradation of 1,1,1-trichloroethane, trichloroethene, trichloromethane and tetrachloroethene in the troposphere to phosgene ($COCl_2$) is discussed in Bächmann and Polzer (1989).

Of the five test compounds, all except 1,1,1-trichloroethane have been classified as substances suspected of exhibiting carcinogenic, mutagenic and tetratogenic potential in addition to nephrotoxicity and hepatotoxicity to varying degrees (Mross and Konietzko, 1991).

1.2.2 Control under the Montreal Protocol

The Montreal Protocol of Substances that Deplete the Ozone Layer is an international agreement made in 1987 (Tierney, 1995). The aim of the Montreal Protocol is to return the loadings of chlorine and bromine to the pre-ozone hole levels of the 1970's by progressively phasing out the production, use and consumption of ozone-depleting substances (Molina and Rowland, 1974). The major developed countries, as the main producers and users of these substances, are expected to take the lead in phase-out.

The Protocol has had two major revisions in 1990 and 1992 which have lead to phase-out dates being brought forward for some compounds (**Table 1.2.2.1**). Note that HCFCs, introduced as replacements to CFCs, are to be phased out as they still contain chlorine. Non-chlorine/bromine containing HFCs are possible replacements, as their atmospheric lifetimes are relatively short and mainly determined by reaction rates with tropospheric hydroxyl radicals.

As can be seen from **Table 1.2.2.1**, two out of the five substances considered in this study, CCl_4 and 1,1,1-$C_2H_3Cl_3$, are covered by the Protocol.

CFCs and CCl_4 all last for decades in the atmosphere, being inert to the common chemical and physical removal processes such as rainout, oxidation and decomposition by UV radiation. This has implications for putative global warming (Jenkinson *et al.*, 1991). Maximum ozone depletion is expected in 2020 assuming the Montreal Protocol is adhered to (Brasseur, 1991). The relatively short atmospheric lifetime of $C_2H_3Cl_3$ results in a lower stratospheric ozone depleting potential than the other compounds controlled by the Protocol. It is, however, included because of its wide range of uses and the consequently larger amounts used. These two compounds will still be in widespread use, at least until 1998.

The Protocol committee has agreed that only three uses are essential for 1996. These are (1) CFCs for metered-dose inhalers for asthma, (2) CFCs for space shuttle solid rocket motor cleaning and (3) a global exemption of all banned substances for laboratory and analytical uses. The justification for the laboratory global exemption is that a number of regulations specify the use of ozone-depleting substances in certain tests and analytical procedures, uses are too varied to list, and the quantities used by each laboratory are small. The users of these substances are expected to develop 'Responsible Care' policies and adopt alternatives wherever possible, such as those listed in **Table 1.2.2.1** that are not controlled by the Protocol, the toxicological hazards of which are generally well documented (Bretherick, 1981).

Table 1.2.2.1 Halogenated solvents and the Montreal Protocol. Not all compounds covered by the Protocol are listed and not all Ozone Depletion Potentials are known. Ozone Depletion Potentials are expressed relative to CFC-12[†] (see table) and are primarily based on degree of saturation and H content.

Compound	**Controlled by Montreal Protocol**	**Phase out date**	**Atmospheric lifetime (years/days)**	**Ozone depletion potential**
$CF_2ClCFCl_2$ (CFC-113)	YES	1/1/96	85y	High
CH_3CCl_3 (1,1,1-tri)	YES	1/1/96	5.4±0.6y	0.1
CCl_4 (CTC)	YES	1/1/96	42y	1.1
CF_2Cl_2[†] (CFC-12)	YES	1/1/96	120y	1.0
CCl_3F (CFC-11)	YES	1/1/96	50±5y	1.0
CH_3Br (MB)	YES	2015	2y	0.7
HCFCs e.g.$CHClF_2$ (HCFC-22)	YES	2015	1-20y	0.0001
HFCs e.g.F_3CH_2F CH_2F_2	NO	None	1.5-55y 14y 6y	None
$CHCl{=}CCl_2$ (TRI)	NO	None	7d	Low
$CCl_2{=}CCl_2$ (PER)	NO	None	140d	0.01
CH_2Cl_2 (MEC)	NO	None	150d	Low

1.2.3 Air pollution meteorology

The atmosphere has a great capacity to dilute, and subsequently remove, toxic air pollutants (Moore, 1983).

Wind effects a dilution (removal of material from the source region) of a component in air proportional to its speed (U, m/s). Therefore at higher velocities, the quantity of material in length x of the emission plume is less and dispersion is greater:

$$\text{Quantity of material in length } x \text{ of plume} = \frac{Q_p}{U}$$

where U = wind speed (m/s)
Q_p = continuous emission rate (units/s)

Pollutant dispersal is seen along the nominal wind direction (Straja, 1994) although the situation is complicated by the effect of surface features such as coasts and high ground, in addition to the height of the source.

The other major mechanism for pollutant dilution is atmospheric turbulence, within the 'mixing' layer (Du *et al.*, 1994). This layer may be anything from metres to kilometres deep. Within this layer, effluent material will disperse in all directions until it reaches the surface or the top of the mixing layer. The rate of dispersion depends on the turbulent energy and the size of the effluent cloud. Turbulence is produced by (1) the stirring of the wind due to the drag of the surface and objects protruding from it and (2) thermal convection currents rising from the surface or descending from the tops of cloud layers. Turbulence is dissipated by viscosity, and atmospheric stability caused by surface cooling or mixing of warm air into the top of the mixing layer.

The dispersive properties of the atmosphere downstream of a given site are further affected by the rotation of the earth which causes wind to blow parallel to the isobars when there is no surface drag (Corolis parameter), time of day (diurnal heating/cooling) and year, type of air mass, cloud cover, surface 'wetness' and the large scale weather situation reflecting degree of free stream flow convergence or divergence (flow inwards to/outwards from the high pressure centres) (Moore 1983; Pasquill and Smith, 1983).

Continuous pollutant and wind variance sampling over a long time frame at small time intervals is required to assess statistically the impact of local sources at monitoring sites and estimate the transport of pollutants over time (Somerville *et al.*, 1994).

Removal and deposition mechanisms

At ambient temperatures, many airborne pollutants may exist as gases or particles (vapour pressures 10^{-4}-10^{-11} atm), or may be distributed between the two phases. The vapour-particle partitioning of compounds is controlled by the vapour pressure and the total suspended particle concentration (Bidleman, 1988; Pankow, 1994). Partitioning may be adsorptive or absorptive (Pankow, 1994).

The amount of pollutant material released from the source which reaches the monitoring site depends not only on dispersion due to the meteorological conditions, but also on the efficiency of atmospheric removal mechanisms. Removal processes operate throughout the depth of the pollutant cloud, and deposition occurs at the surface. The total pollutant flux and the vertical profile will be altered by removal and deposition (Grünhage *et al.*, 1994). Four main removal mechanisms exist:

(a) *Dry deposition* : diffusion to or impaction and sedimentation on the earth's surface. The deposition rate is controlled by chemical/biological interactions between surface and material, and wind and atmospheric. Particulate, aerosol and gaseous dry deposition processes occur (Bidleman, 1988);

(b) *Wet deposition (rainout)* : within-cloud diffusion to or impaction on cloud or spray droplets. Permanent removal will only result if the droplets are impacted themselves or swept up by washout. If material is present as gas or particles which do not become condensation nuclei, there is usually a delay before material is taken into the cloud water. Vapour scavenging is favoured by a low Henry's law constant (Bidleman, 1988);

(c) *Wet deposition (washout)* : below cloud diffusion to or impaction by raindrops, hailstones or snowflakes. Permanent removal will only be effected if these species reach the ground without evaporating;

(d) *Occult deposition* : cloud or fog drops impact on vegetation or fall to the ground. The rate of removal of condensation nuclei is up to an order of magnitude faster than in unsaturated air due to the much larger fall velocities and inertia of activated droplets. Soluble gaseous pollutants and smaller aerosol particles may be captured by cloud drops in a similar way. The cloud droplets in turn are easily captured by the tall, aerodynamically rough morphology of trees, with forest geography and altitude greatly influencing the form of pollutant and deposition. Wind plays an important part in the process and hill fogs produce more deposition than radiation fogs (Dollard and Unsworth, 1983).

Both vapour phase and particulate phase species can be removed by dry and wet deposition fluxes (Bidleman, 1988). The relative contributions of dry and wet deposition, and different sized species, vary with climate and vegetation forms (Burkhardt and Eiden, 1994).

1.2.4 Uptake of air pollutants by forests

Pollutants taken up by forests generally fall into one of two categories (1) those taken up by the leaf and branch surfaces either via atmospheric removal and deposition processes or direct adsorption e.g. into epicuticular wax, or (2) gases which enter via the stomata and are not absorbed readily by external foliar surfaces (Koziol and Whatley, 1984).

Pollutants in the first group include the gases HCl, HNO_3, C_1/C_2 chlorinated hydrocarbons, and NH_3, SO_2 and cloud droplets in some circumstances. The rates of deposition on forests for these substances are close to the maximum value provided for atmospheric processes assuming constant fluxes of momentum, heat and mass (Grünhage *et al.*, 1994), and are generally much larger than those for shorter vegetation (DoE, 1993). The concentrations of many major pollutants are higher in cloudwater than rainwater (Crossley *et al.*, 1992) which can lead to multiple chemical stress (Vong and Guttorp, 1991). Enhancement of acidity in cloud droplets can be seen when physical changes occur at high altitudes and the water droplets pick up extra oxidants such as SO_2 and NO_x (Bowman and Likens, 1979). In addition, NO_2 may induce the generation of photooxidants in cloud water such as hydrogen peroxide (Frank and Frank, 1985; Kettrup *et al.*, 1991). The uptake from air and partitioning of the lipophilic C_1/C_2 chlorinated hydrocarbons into epicuticular wax is discussed further in Chapters 2,4 and 7.

The second group includes O_3, NO_2 and SO_2 (in the absence of NH_3). These pollutants, being under the control of stomatal conductance, display marked diurnal and seasonal cycles and the rates of dry deposition in forests are similar to those for shorter vegetation.

A resistance analogue for pollutant uptake by the forest canopy is shown in **Figure 1.2.4.1**. The sum of all the resistances, r_t, is given by the sum of all the atmospheric resistances (r_a+r_b) and canopy resistance (r_c). The former includes the still air or boundary layer which depends on a number of factors including wind speed and leaf properties such as shape, size and orientation. Canopy resistance is determined by up to four parallel paths, stomata (r_{d1}), external plant surfaces (r_{d2}), surface water (r_e) and soil (r_{d3}). Once inside the leaf, further resistance may be offered by the mesophyll (not shown in the figure). This mesophyll resistance consists of a number of different components: the substomatal air space, the extracellular fluid, the cellulose cell wall, the plasma membrane, the cytoplasm and organelle envelope membranes before the reaction site is reached.

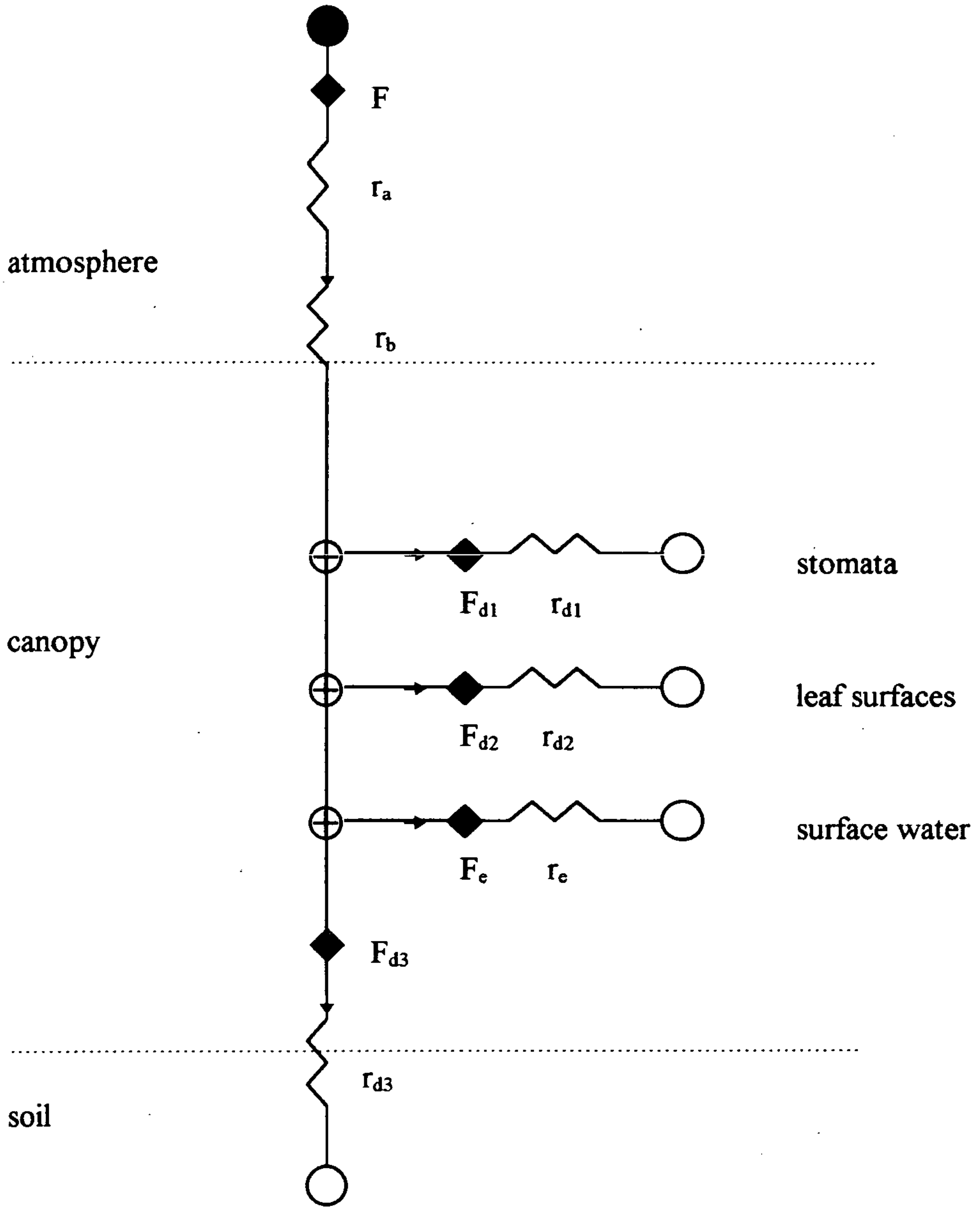


Figure 1.2.4.1 A resistance analogue for pollutant uptake by forest canopies. Descriptions of the resistances (r) are given in the text, the corresponding fluxes are denoted by the prefix F. After DoE report, 1993.

The trans-cuticular pathway (r_{d2}) involves solubilization of gases in the lipids of the polymeric cuticle membrane (Baker, 1982) and transfer is enhanced for most group 1 (and some group 2) compounds by a layer of water (r_e) on the surface of the leaf (Burkhardt and Eiden, 1994). Some group 1 species such as nitric acid exhibit no surface or canopy resistance, and the rate of deposition depends only on rates of turbulent and diffusive transfer to the trees. The driving force behind the transport of ions across the cuticle is the concentration gradient between the inner and outer solutions modified by the electrical potential across the cuticular membrane (Burkenhardt and Eiden, 1994). This type of mechanism is particularly important when considering the uptake of acidic species by the cuticle.

As is shown in **Figure 1.2.4.2**, the bulk of plant cuticle is made up of cutin, a strongly cross-linked polymer network (Holloway, 1982). It has been shown that removing the external wax layer from cuticles results in an increase of gas and water permeability of up the three orders of magnitude (Kerstiens, 1993 *in press*). This layer is therefore a very effective barrier, and movement of gases through it can be thought of as the diffusing of molecules through a polymeric array containing crystalloid obstacles (Schönherr and Riederer, 1989). The elongation of the mean diffusion path by collision reduces solute mobility (thermal energy) and transport rates.

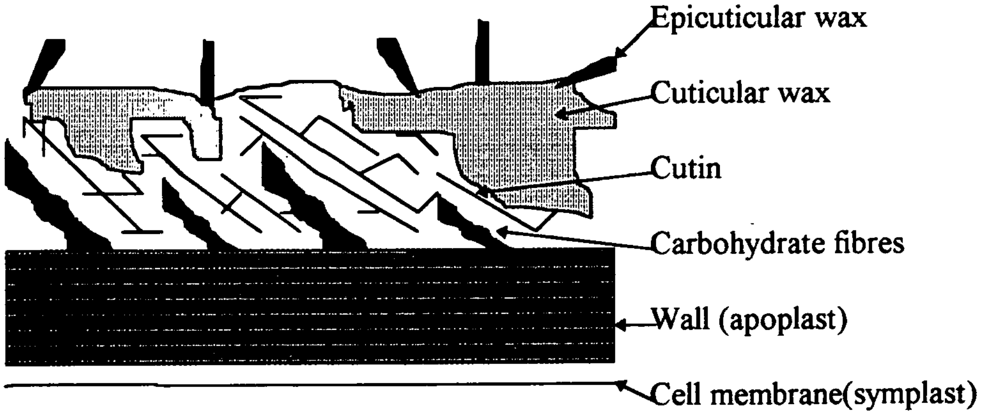


Figure 1.2.4.2 Idealised section through the cuticle. After Price, 1982.

Some regions of the wax are partly amorphous, which will govern cuticular permeability. The polysaccharide reticulum found in most cuticles also affects water uptake by increasing permeance (Kerstiens, 1993 *in press*), possibly by providing diffusion pathways with special properties (Holloway, 1982). The molecular composition, distribution and lipophilicity of epicuticular waxes are discussed further in Chapter 5, and detailed descriptions are given in 'The Plant Cuticle' edited by Culter, Alvin and Price (1982).

1.2.5 Reaction mechanisms

Several mechanisms have been proposed for the type of forest decline described. These include correlating the yellowing of spruce needles with loss of magnesium, possibly as a shoot-mediated effect (Cape *et al.*, 1988; Innes, 1991; Ulrich, 1990); other possible effects of acidification on forest soil and foliage (Ulrich 1990, Vong and Guttorp, 1991); and light-accelerated pigment bleaching reactions (Frank and Frank, 1985).

Superoxide, hydrogen peroxide and hydroxyl radicals are considered to be initiators of pigment bleaching (Osswald and Elstner, 1987; Ruth and Weisel, 1993). They are formed when photooxidants such as ozone react with alkenes, such as ethene produced under stress by plants, and natural monoterpenes (Isidorov *et al.*, 1985), and cause 'over-reduction' conditions in the electron transport chain (Dechaux *et al.*, 1994; Möller, 1988). Such species are converted to water in the chloroplast detoxification cycle at the expense of glutathione and other counteracting metabolites (Osswald and Elstner, 1987). The ratio of reduced to oxidised glutathione has been found to be higher at damaged sites and when ozone levels are high, which may be indicative of oxidative stress (Schmieden *et al.*, 1993).

The current thinking on the cycling of chlorinated C_2 hydrocarbons and trichloromethane in the atmosphere and terrestrial environment is summarised in **Figure 1.2.5.1**.

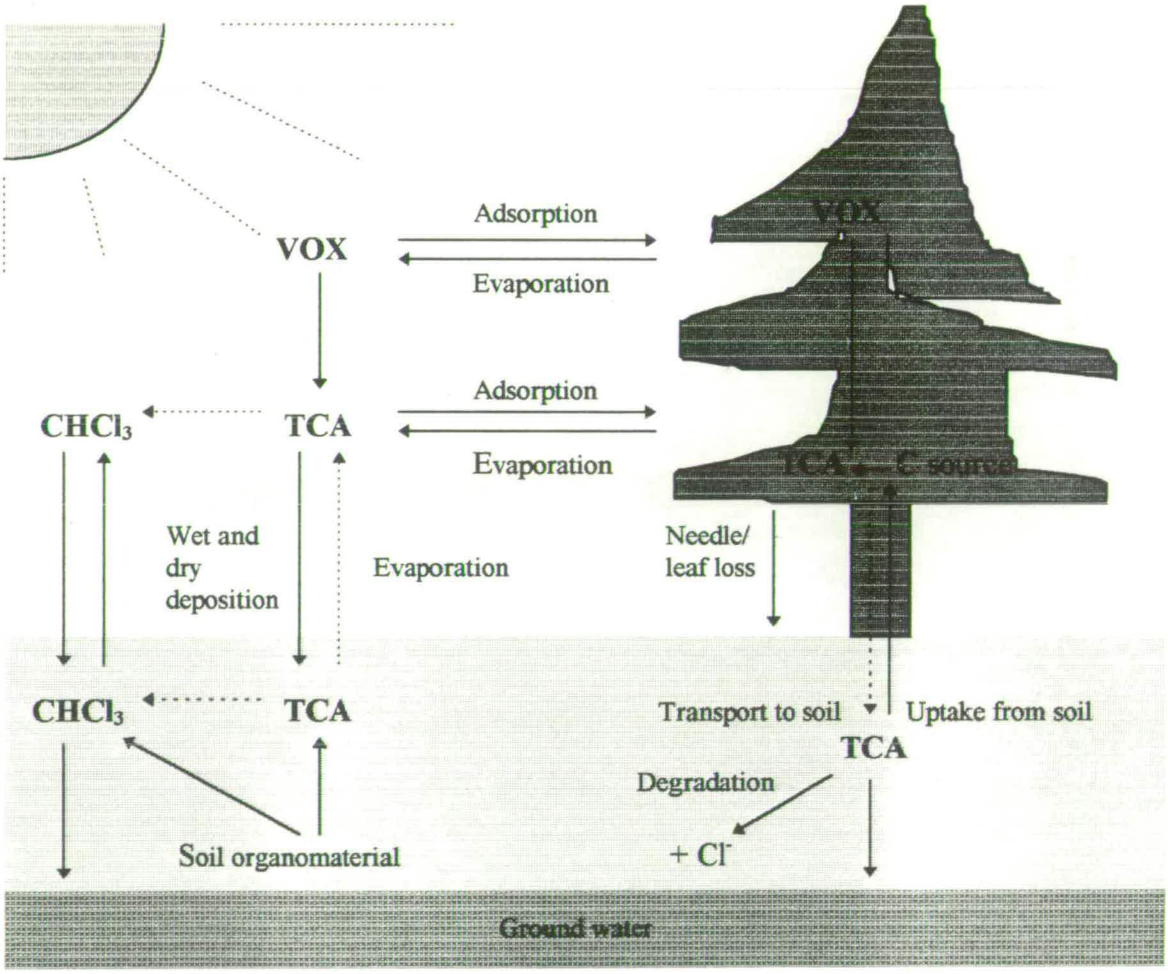
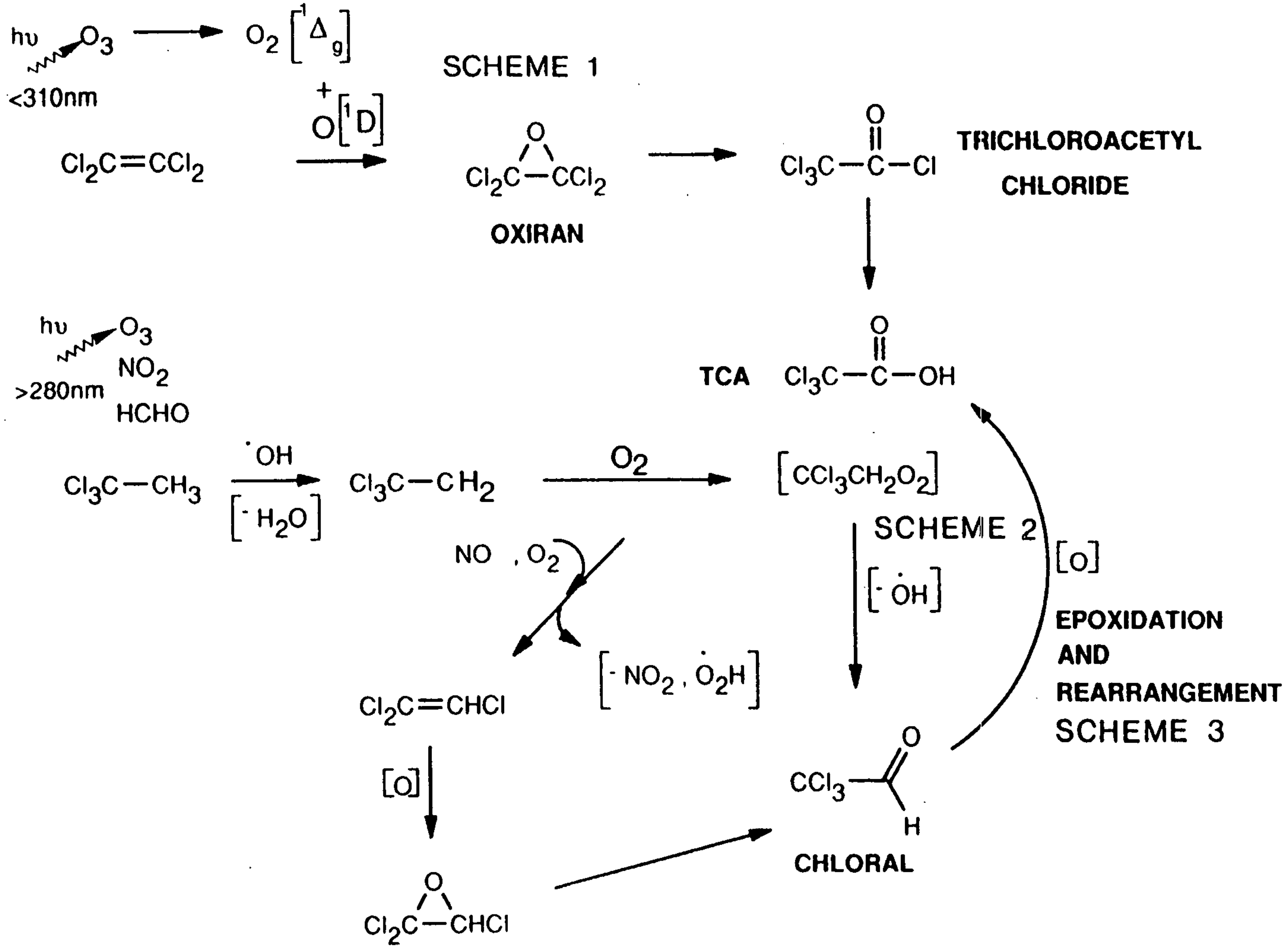


Figure 1.2.5.1 The possible cycling of C_2 chlorinated hydrocarbons, trichloromethane and TCA in the atmosphere and terrestrial environment. (VOX=volatile chlorinated C_2 hydrocarbons). After Hoekstra and de Leer, 1995.

It has been suggested that trichloroacetic acid (TCA, CCl_3CO_2H) and other chloroacetates are formed by the photooxidation of C_2Cl_4, C_2HCl_3 and CH_3CCl_3 (and phosgene, $COCl_2$) in the atmosphere (Frank 1991; Fuchs and Bächmann, 1987). Large amounts of TCA have been detected in forest air, soil, water and foliage (Frank *et al.*, 1994; Plümacher and Renner, 1993 *in press*).

The mechanism for TCA formation probably proceeds through rearrangement of the primary tetrachlorooxiran ring (Scheme 1) or hydroxyl radical attack and oxidation of chloral (Scheme 2). The third mechanism proposed is via epoxidation and rearrangement (Scheme 3). Tentative mechanisms for mono-chloroacetate (MCA) formation have been proposed by Frank *et al.* (1994).

TCA and MCA are well known for their herbicidal properties, and as such are highly phytotoxic (Ashton and Craft, 1973; Foy, 1969). TCA action is possibly through targeting the enzymes involved in coenzyme A synthesis leading to degradation of epicuticular wax and increased water permeability and thereby a weakened defence against fungal infection (Hilton *et al.*, 1959; Juniper, 1959). MCA may exert toxicity in a similar manner to fluoroacetate by altering the mitochondrial citric acid cycle (Debus and Schröder, 1989).



Reaction schemes : Photochemical activation of chlorinated solvents

The mechanisms proposed above are not, however, universally accepted. For example, atmospherically degraded chlorinated pollutants would mainly end up as phosgene and chloride (Bächmann and Polzer, 1989; Hoekstra and de Leer, 1995). In addition, TCA arising only from such processes cannot account for the large amounts found in conifer needles in a variety of locations (Frank *et al.*, 1992; Juuti *et al*, 1993; Norkorpi and Frank, 1993 *in press*). Three possibilities present themselves, either;

(1) Atmospheric processes are not fully understood, TCA dissolves in rainwater at concentrations up to 1µg/L (Fuchs and Bächmann, 1987; Plümacher and Renner, 1993 *in press*) but uptake into needles is not expected to be large due to the very low octanol-water partition coefficient of TCA; or

(2) TCA produced in soil, possibly from chlorinated hydrocarbons present at concentrations of up to 4.5µg/m^3, is taken up by the roots of trees in the transpiration stream and stored in the needles (Renner and Mühlhausen, 1989); or

(3) Quantities of TCA are formed *in situ* in foliage from chlorinated species enriched in epicuticular wax and irreversibly trapped within the needle interior (Schroll *et al.*, 1994). The three major C_2 chlorinated hydrocarbons can be converted to TCA by cytochrome P-450 dependent metabolism.

Chapter 8 contains further discussion on TCA formation and possible effects.

Photochemical activation of chloroethenes leading to the destruction of photosynthetic pigments has been suggested as another possible mechanism for the observed foliar bleaching in conifers (Frank and Frank, 1985). Chloroethenes may be activated by UV radiation chloroethenes to excited triplet states and radicals (Cl•) leading to formation of phytotoxic compounds such as chloroacetyl chloride and phosgene (Schemes 1 and 2). Photochemically excited photosynthetic pigments may therefore be expected to react with chloroethenes with concomitant destruction, the analogous reaction being the addition of chloroethenes to electronically excited dienes and polyenes. Chlorophyll-a and β-carotene show most bleaching, concomitant with strongly depressed needle concentrations and high UV light levels (Frank and Frank, 1986). Radiation in the UV region is only found at high altitudes because it is strongly attenuated by dust-laden, semi-urban and urban atmospheres.

1.3 Study objectives

The project focused on the spatial distribution of the environmentally significant C_1/C_2 chlorinated hydrocarbons $CHCl_3$, $C_2H_3Cl_3$, CCl_4, C_2HCl_3 and C_2Cl_4 in the foliage and air of three forests in northern Britain. Although some data are available for central European sites (Frank and Frank, 1990; Plümacher and Renner, 1993 *in press*), no group has reported concentration data for these compounds in UK forests. The tree species monitored was Scots pine (*Pinus sylvestris* (L.)) due to its widespread distribution in northern Europe, and the presence of suitable stands at each forest site.

Passive air samplers were developed for the air monitoring programme (Brown *et al.*, 1992; Gilli *et al.*, 1992). In order to understand the relationship between chlorocarbon concentrations in *Pinus sylvestris* needles and ambient concentrations in air, a knowledge of the partition coefficient characteristics is required (Schreiber and Schönherr, 1993). Published literature values are exclusively for spruce needles (Figge, 1988; Frank and Frank, 1989).

Analytical techniques were developed which used the available equipment to its maximum potential. Controlled environment studies were outside the scope of this project (Ruth and Weisel, 1993).

The objectives of this study were:

(1) Development and optimisation of a capillary gas chromatography-electron capture detection method for determining levels of these environmentally significant C_1/C_2 chlorinated hydrocarbons in *Pinus sylvestris* (L.) needles and air sampler wax to 0.1ng/g levels.

(2) To establish a data base to describe chlorocarbon status in *Pinus sylvestris* (L.) needles of some forests in the UK between March 1993 and June 1994. One rural and two industrial sites were used. The rural site was at Banchory forest to the east of Aberdeen in Grampian region, Scotland. The forests close to industry were Ponsonby Tarn forest near the nuclear installation at British Nuclear Fuels plc. Sellafield, West Cumbria in England, and Devilla forest across the Firth of Forth from the petrochemical complex at Grangemouth, Central Region, Scotland. The Sellafield nuclear reprocessing site is predominantly a low height source for these substances, the Grangemouth chemical complex is a mixed height source.

The hypotheses to be tested were :

- (i) Amounts found in UK conifer needles are similar to published data from central European forest sites;
- (ii) Larger amounts of chlorinated solvents are seen at industrial rather than rural sites;
- (iii) Concentrations decrease at Ponsonby Tarn forest with horizontal distance (west to east) from the nuclear installation at Sellafield in West Cumbria;
- (iv) A vertical profile exists through the canopy at Devilla forest near petrochemical industry at Grangemouth;
- (v) The concentrations found are linked to the predominant wind direction and speed measured during the period preceding sampling;
- (vi) Larger amounts are found in older needles due to accumulation over time.

(3) To use air sampler data from the three sites to assess actual amounts taken up by needles from the air when related to partitioning into needles and wax, and to confirm partition ratio assignments.

(4) To develop laboratory based apparatus to generate standard atmospheres at higher than ambient concentrations and a GC/ECD method to measure chlorinated solvent partition ratios into wax and whole needles at these high air concentrations.

(5) To develop a GC/MSMS method for complementary qualitative assessment of chlorinated solvent concentrations in needles.

1.4 Layout of thesis

Analytical methods used to measure volatile chlorinated solvent concentrations in air and foliage are reviewed in Chapter 2. The passive air sampler design used, and the development of the chosen analytical method are described. The choice and location of sampling sites are considered together with sampling frequency. Chapter 3 gives a brief introduction to GC theory with further details of GC method optimisation. Complementary GC/MSMS results from development work at the Scottish Agricultural Scientific Agency (SASA) at Edinburgh are presented.

The studies to measure chlorinated solvent partition ratios into whole *Pinus sylvestris* (L.) needles and a needle wax extract at high air concentrations are described in Chapter 4. The results are compared with literature examples for spruce (*Picea abies*) and correlations made with compound octanol-water partition coefficients (K_{ow}), Henry's law constant (H) and the air-water partition coefficient (K_{aw}).

The statistical methods used to treat the calibration data and results from the forest sites are described in Chapter 5. The blank-corrected needle results are discussed with reference to distribution trends in Chapter 6. Median concentration values across all time at each forest are presented and compared with literature values, in addition to seasonal concentration variation and within/between-site variations.

In Chapter 7, the relationship found between compound concentration in needles and air sampler tube wax is discussed with reference to the laboratory-determined partition coefficients and reported literature data. The conclusions from the study are presented in Chapter 8, together with ideas for future work.

Chapter 2

Experimental : Sampling methods and preliminary investigation

We see 'the strong hold which in all ages chemistry has had on the imagination. If in Shakespeare we find nature idealised into poetry, so through the creative power of a profound yet observant meditation in chemistry...we find poetry, as it were, substantiated and realised in nature: yea, nature itself disclosed to us...as at once the poet and the poem'.

Samuel Coleridge

2 EXPERIMENTAL : SAMPLING METHODS AND PRELIMINARY INVESTIGATION

2.1 Introduction

The accurate determination of both airborne and bioaccumulated chlorinated hydrocarbons demands that choice of sampling location, frequency of sampling, sample collection, sample storage, sample transfer to analytical measuring device, detection and calibration steps are of equal importance. Each step requires optimisation with respect to the other components of the procedure and variation in any one step will affect all the others (Hanson and Perry, 1986) (**Figure 2.1.1**).

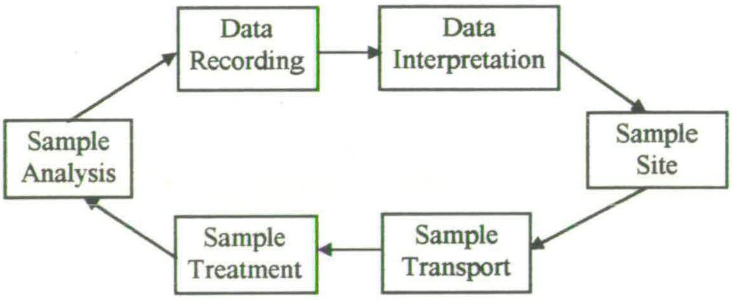


Figure 2.1.1 Air monitoring concept system

We need to be sure that unusually high or low values, particularly of atmospheric concentrations, are real and not simply due to sampling or analytical artefacts.

Several analytical methods for volatile chlorinated solvents claiming high reproducibility, simplicity, cost-effectiveness and accuracy have been presented in the literature (Bloemen and Burn, 1993; Diezel *et al.*, 1988; Frank and Frank, 1988a).

Most are based on gas chromatographic separation (GC) and electron capture detection (ECD), some with mass spectroscopy (MS) for confirmation (Clement *et al.*, 1993; Sharp *et al.*, 1992 see further discussion in Chapter 3). No single method of sampling volatile organic compounds (VOC's) in general, either atmospheric, indoors, or bioaccumulated, has become a standard or reference method, although the US Environmental Protection Agency methods are routinely used for the analysis of a wide variety of volatile analytes in different matrices (Hanson & Perry, 1986). Similar guidelines have been published in the UK by bodies such as MAFF (Allchin *et al.*, 1989).

2.2 Air sampling method

2.2.1 Review of air sampling methods

All methods of measuring airborne chlorinated solvents have their advantages and limitations. Many are based on active (pump) air sampling techniques, often adapting a filter-adsorbent system (**Figure 2.2.1.1**). An appropriate filter in the air entry allows sampling for the particulates, and an adsorbent selectively traps the components of interest in the gaseous phase (Hewitt, 1991; Kaupp and Umlauf, 1992). Desorption (thermal or solvent) from this adsorbent is followed by chromatography for analysis (Frank and Frank, 1988a).

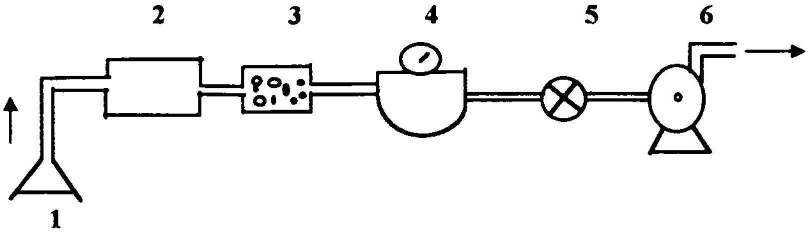


Figure 2.2.1.1 Air sampling train (from Hewitt, 1991). **1**, air entry with filter; **2**, separation and concentration; **3**, pump protection; **4**, air volume measurement, **5**, flow control; **6**, pump.

Pump-based filter-adsorbent systems can suffer from sampling artefacts such as chemical transformation of compounds passing through the filter. Adsorption of gaseous substances on deposited particles or the filter ('blow-on'), and 'blow-off' (temperature or concentration change during sampling leads to movement of particle- or filter-bound compounds to the adsorbent) can lead to over-estimation of the particle and gaseous phase concentrations respectively. Alternative active sampling techniques such as adapted high volume air sampler filters to reduce sampling time while maintaining pressure and flow rate (Hart *et al.*, 1992), denuders (Hewitt, 1991) and diffusion denuders (Kreiger & Hites, 1992) for the collection of semi-volatile organics (e.g. PCB's) can offer improvements. Such modifications are important when concentrations are so low as to require long sampling times and large sampling volumes (>200m^3).

The second main technique of determining airborne chlorocarbon concentrations in ambient atmospheres uses passive sampling and is less widespread. Passive, or diffusive, samplers were initially developed during the 1970's (Palmes and Tomczyk, 1979) for the measurement of personal exposure to airborne contaminants in the workplace, typically at >1ppm, as a time-weighted average. More recently, passive samplers have been applied to the monitoring of low concentrations of organic vapours in both indoor (Brown *et al.*, 1992) and ambient air (Koutrakis *et al.*, 1993).

The passive process is comparable with the temporal and spatial scale of interest (Cao and Hewitt, 1991). Passive samplers consist of a diffusion barrier e.g. air, a collection medium and a sampler case (**Figure 2.2.1.2**). The primary feature of a passive sampler depends on the collection medium (Lee, 1992). 'Liquid' and 'solid' collection media can be identified, the former often coated on a mechanical support and using chemical reaction for adsorption, and the latter using chemical or physical adsorbent properties. Adsorbates are recovered, as in active sampling, by solvent extraction (Ogden and Malolo, 1992) or thermal desorption (Van den Hoed and Halmans, 1987).

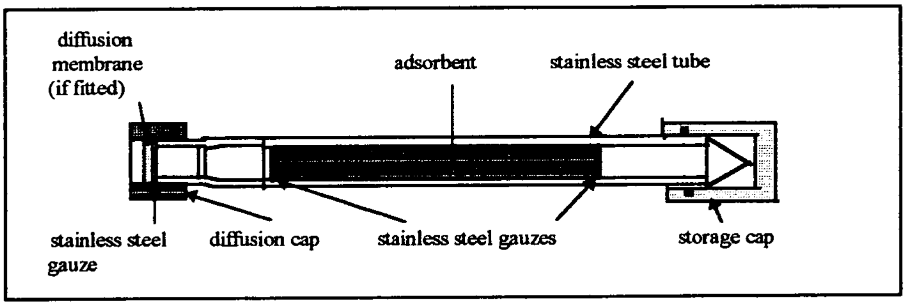


Figure 2.2.1.2 Schematic of a typical passive tube-type air sampler. (From Perkin Elmer, 1992).

Passive samplers are an attractive option because of their simplicity and cheapness. In addition, they do not require a power supply like conventional pump sampling techniques.

The third main technique used is collection in evacuated electropolished aluminium or stainless steel canisters, transfer to an adsorbent and chromatography (Mueller and Oehme, 1990). Other general techniques exist including the use of a sweep gas to displace the headspace of a liquid sample into the detection system (Harper, 1985; Hewitt *et al.*, 1992; Voice and Kolb, 1993).

For low concentrations, this technique can be used to 'strip' volatiles trapped and pre-concentrated in solid adsorbent beds (Burger *et al.*, 1990). Purge and trap (dynamic headspace) methods allows more rapid extraction of volatiles than static headspace analyses, but are difficult for very volatile species such as vinyl chloride and chloromethane. The gas is purged through the liquid before passing through an adsorbent trap. In these last two techniques, the volatility of the host matrix is important. The adsorbent trap will become overloaded with the sample medium if the purge gas becomes loaded with large quantities of the host material.

Solid samples may be directly desorbed into an adsorbent or cryogenic trap, although supercritical fluid extraction (SFE) (Dale, 1991), supercritical fluid chromatography (SFC) and headspace solid-phase microextraction (Zhang and Pawliszyn, 1993), as an adaptation of solid-phase extraction, have superseded such techniques. The solvent-free sample preparation technique of headspace solid-phase microextraction uses a fused silica fibre, coated with a polymeric organic solid or liquid. When inserted into the headspace, volatilized organic analytes are extracted and concentrated, desorbed and analysed by GC (GC/MS). The technique is based on the equilibrium partitioning of the analyte between the fibre coating and aqueous solution.

This review will briefly consider the main methods based around active sampling and canister sampling, as used by other authors, before considering passive sampling techniques. The advantages and limitations of all three techniques are considered, and the choice of passive sampling as the preferred technique is justified.

Active air sampling methods

The preferred method is analyte pre-concentration on an adsorbent-filled cartridge by a pumped sampling process before thermal desorption/cryogenic focusing (or equivalent; Frank & Frank, 1988b) or solvent extraction (Bauer, 1991) and chromatography. Enrichment in cold traps, and collection in a gas loop of a 6 (or 10) - port valve with direct injection to chromatography (on-site) (Frank *et al.*, 1991b; Hinsham and Grosjean, 1991) are other popular methods. The relatively low concentrations of organic contaminants in ambient air often necessitate a concentration step before chromatographic analysis (Sharp *et al.*, 1992), although, as indicated above, sample collection may itself be a pre-concentration process in which analytes are stripped from the air.

The main disadvantages of cold trapping are the accumulation of water vapour (Rudolph *et al.*, 1986). Evacuated canisters are subject to contamination of the walls and therefore are of finite lifetime, in addition to being bulky and expensive to transport (Perkin Elmer, 1992). Analyte adsorption and enrichment on solid adsorbents, either on-site or with a central instrument, is preferred despite the costs of cryogenics, thermodesorbers and electronics (Frank *et al.*, 1991b).

Incorrect choice of sorbent may result in analyte loss during thermal desorption. The solid adsorbents Tenax® (Sturges *et al.*, 1992), activated charcoal, graphitized carbon (e.g. Graphtrap, Carbopak®, Carbotrap®) or multisorbent systems appear to be favoured (Frank *et al.*, 1991b; Gulyas and Hemmerling, 1990; Sykes *et al.*, 1980), although silica gel, XAD-resins, Chromosorb® resin (polyaromatic cross-linked with high adsorption capacity) and other porous polymeric materials have been used, e.g. Porapack®, in addition to inorganic materials such as Florisil® or molecular sieves. Such adsorbents have the advantages of being relatively inexpensive, being re-useable through reactivation, showing low background contamination and displaying great versatility.

Tenax® is a porous polymer of 2,6-diphenyl-*p*-phenylene oxide and displays a broad range of trapping compounds and effective adsorption/desorption efficiencies. Tenax® has the advantages over other porous polymers of a low affinity to water and high chemical stability, thus thermal desorption is promoted relative to solvent desorption and low capillary column bleed has been demonstrated during chromatography, particularly by Tenax TA®.

Disadvantages of Tenax® include polymeric degradation to benzaldehyde and acetophenone on exposure to oxidants (e.g. atmospheric ozone (Hewitt, 1991) and acids (Ciccioli *et al.*, 1986)), relatively low breakthrough volumes († see overleaf) for low molecular weight volatile organic compounds, and artefacts that can occur during sampling, storage or sample desorption (Rudolph *et al.*, 1986).

Table 2.2.1.1 compares the breakthrough volumes of four popular adsorbents used for chlorinated solvent sampling. Tenax® displays a large difference between the most and least retained chlorocarbons and the BTV's for CH_3CCl_3 and CCl_4 are small. The higher capacity of charcoal for the more volatile components is advantageous. High BTV values may mean that adsorbed chlorocarbons may be more difficult to desorb. The limiting factors for quantitative sample enrichment are the sample amount necessary for detection and the smallest BTV of the compounds of interest. Safe sampling volumes (Brown and Purnell, 1979) for a tube of defined dimensions containing a certain adsorbent are less than BTV's at a given temperature, vapour concentration and flow rate.

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† **Break-through volume (BTV)**: The maximum volume of air which can pass through an adsorbent with complete retention of the compound of interest. The BTV is dependent upon concentration, temperature, other gases present (e.g. H_2O), flow rate, particle size, trap composition and dimensions. The BTV is determined by installing the cartridge as a GC column, and derived either directly at the temperature of sampling or, for less volatile components, by extrapolation (Frank and Frank 1988a). The **retention volume (RV)** is the point at which a single injection of vapour emerges from the adsorbent tube and is measured at the chromatographic peak maximum. The approximate BTV = RV/2 (Maier and Fieber, 1988).

The breakthrough of trichloroethene (at <13L/g adsorbent) is seen in **Figure 2.2.1.3**, (Heavner *et al.*, 1992) with 100% loss from the back cartridge of a spiked tandem multisorbent system (Tenax® /Carbotrap®) at volumes greater than 40L. The other chlorocarbons had breakthrough at >50L/g adsorbent.

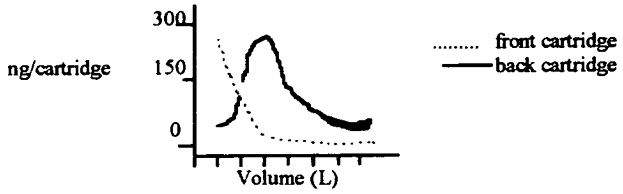

Figure 2.2.1.3 Breakthrough of trichloroethene. Effect of sample volume on front tube recovery from spiked tandem multisorbent cartridges.

Table 2.2.1.1 BTV's (L) of some chlorinated solvents on four adsorbents at 36°C; adsorbent bed 90 x 3mm, charcoal 41 x 3 mm; N= number of theoretical plates (from Frank and Frank, 1988a).

	$CHCl_3$	CH_3CCl_3	CCl_4	C_2HCl_3	C_2Cl_4
Tenax TA, 89 mg, 60-80 mesh N= 5-50	0.44	0.04	0.04	1.14	4.47
Charcoal SK-4, coated with 1% SP-2100, 68mg, 60-80 mesh N= 0-30	196	62	546	58000	-
Mixture of 90% Tenax TA + 10% SP-2100-coated charcoal SK-4,110mg N= 5-30	11	51	59	127	450
Graphtrap, 500mg, N=20-70	0.2	0.5	0.5	1.2	24

In addition to adsorbent type and BTV's, cartridge capacity, desorption volumes, blank levels and detector linearity range and sensitivity must be considered together when the trap is to be analysed directly by GC. A suitable sampling size, carrier gas flow and column film phase/thickness must be chosen. Capillary columns are preferred for GC, having better separation and resolution (see Chapter 3). The electron capture detector is particularly sensitive for electrophores and is able to detect to sub pg/injection levels equivalent to $\mu g/m^3$ of chlorocarbon in air.

In all pumped systems, at such low concentrations, there is an increased potential for changes in concentration over the sample period and concomitant artefact formation. Degradation in the chemical composition of the sample is possible, and in addition, physical and chemical sorption on tube walls or deposited aerosols therein, humidity and released chemical interferents from tube walls can be problematic - teflon is not inert for these purposes! (Hewitt, 1991). Temperature effects on materials used in pumps are important. The fast compression process ('quasi-adiabatic') can cause decomposition or rearrangement of trace gases which are less thermally stable (Rudolph *et al.*, 1986). Halocarbons are ubiquitous contaminants in almost every matrix - in all solvents, most carrier gases and the organopolymers used as sealing materials. Even 'metal-bellows' pumps can cause contamination of the sample with low molecular weight alkenes and halocarbons.

Contaminants are particularly problematic at these low concentrations when cryogenic trapping is involved (Frank, 1988b). Cryogenics in general, whether in traps or focusing, are technically demanding, expensive and time-consuming for field analyses or automated operation, requiring large amounts of coolant and energy. When using pumps, the capacity of the pumping system is important, varying sampling times and flow rates will ensure that sufficient mass is collected for analysis. The minimum period of sampling can be limited by pump capacity or halocarbon/air separation efficiency (Hewitt, 1991).

The upper and lower amounts of analyte (i.e. sample volume) are limited by three main factors ;

(1) The linearity of the electron capture detector (see Chapter 3);

(2) The gas chromatography, as capillary columns limits are lower than packed columns (Frank, 1988b), and in particular;

(3) The contaminant levels of the blanks. The minimum sample size is limited by blank levels, and the effective lower limit of the ECD will be raised from typical levels of 150fg (CCl_4) - 500fg (C_2HCl_3) with a signal to noise ratio of 5, to pg levels (Frank, 1988b) when contaminants are present.

The different extraction methods have various advantages and limitations depending on nature of sample, locations to be monitored and the range of concentrations. The enrichment/desorption process of thermal desorption and cryogenic focusing as a gas extraction technique offers the advantages of direct liberation of analytes from matrix to gas phase of chromatography (Cole and Woolfenden, 1992). The method can be automated (Tsetsi *et al.*, 1989) and high enrichment ratios with accompanying increases in analytical sensitivity can be obtained. Disadvantages include the cumbersome nature of cryogenic traps and associated water collection, although stationary-phase focusing at ambient temperature (microtrap or PoraplotQ; Frank and Frank, 1990; Sharp *et al.*, 1992) is an alternative method to cryofocusing. Careful assessment of BTV's is necessary to ensure that those for the compounds of interest on the adsorption cartridge exceed the volume of air taken as a sample, that thermodesorption is complete and post-cryogenic recovery is 100% (Frank and Frank, 1988b). Traces of polluted laboratory air can be taken up during cartridge transfer to the thermodesorber (Frank and Frank, 1988a). Artefacts often arise, particularly during analysis of volatile species as thermally labile compounds are broken down. A major disadvantage is the expense of the equipment.

The thermodesorption process can be broken down into three stages : sampling, desorption to cold trap and flash heating. Passive or active sampling transfers analytes from host matrix, i.e. air, to the adsorbent bed. The adsorbent is heated and purged with an inert gas. Trapped volatiles are desorbed into the elution gas and swept from the adsorbent bed into a cold trap with a low dead volume (20µL). Organic volatiles are frozen out and concentrated. Flash heating releases the analyte from the cold trap to chromatography, and the detector (Noy *et al.*, 1987). Relative standard deviations of ±10% have been reported for replicate analyses under optimised conditions (Frank and Frank, 1988a).

Solvent extraction techniques, based on the relative solubilities of the analyte in extracting solvent and sample collection adsorbent, have been widely used in the removal of volatile components from an innumerable variety of matrices (Dale, 1991; Heavner *et al.*, 1992; Sykes *et al.*, 1980). This technique allows replicate analyses of samples, unlike thermal desorption, with concomitant reduction in artefact formation. There are disadvantages to the technique which must be recognised. Detection limits can be restricted by the dilution effect associated with the use of relatively large solvent volumes, and solvent extraction will re-dilute enriched components. The solvent may also introduce contaminants into the system, as will exposure to laboratory air, or interfere chromatographically with the analytes of interest.

Ultimately all these factors determine the type and amount of adsorbent and the cartridge dimensions. Tenax®/1% polydimethylsiloxane-coated charcoal has been used as an adsorbent, having a sufficiently large specific capacity for C_1/C_2-chlorocarbons but not the more volatile fluorochlorocarbons (Billings and Bidleman, 1980). This adsorbent is better for analytes with boiling points over 100°C. The retention times at ambient temperature using active air sampling are of the order of a few hours to days and the minimum relative difference of breakthrough volume should be selected for (Maier and Fieber, 1988). The smallest BTV will be limiting.

Passive air sampling techniques

Two different sampler geometries have been used, badge-type (e.g. OVM#3500, Adkins and Henry, 1993) and tube-type (e.g. Perkin Elmer diffusion tubes). Disadvantages of the badge-type sampler are;

(1) Inappropriateness for thermal desorption. Solvent extraction methods (e.g.CS_2) are used for analyte recovery followed, in general, by gas chromatography with an appropriate detector. The dilution effect and analysis of only a small aliquot (typically 0.01%) of extract result in lower sensitivity, although desorption solvent additives, such as 10% amyl alcohol or 5% 2-(2-butoxyethoxethanol), can improve desorption of polar compounds (Adkins and Henry, 1993).

(2) The high area : length ratio results in high uptake rate, and a minimum face velocity (wind speed) of 0.05-0.1 m/s is needed to ensure there is sufficient air flow over the sampling surface (Appendix 1).

Several patents have been taken out, though, for novel badge-type passive samplers (Adkins and Henry, 1993). An example is a film badge biosensor, for PAH's amongst other hazardous environmental chemicals, which mimics a biological response mechanism related to tumour initiation in carcinogenesis. Specific chemical passive dosimeters use reagents to form derivatives for analysis such as porous PVC discs to monitor phosgene ($COCl_2$) (Adkins and Henry, 1993). Despite its inability to retain very volatile organic chemicals, e.g. vinyl chloride and methylene chloride, Tenax® has been adopted for passive badge use (Coutant *et al.*, 1986).

Tube-type diffusion samplers are amenable to both solvent extraction and thermal desorption, the latter allowing whole sample transfer to the detector resulting in increased sensitivity. They have a much lower minimum face velocity of 0.001 m/s, promoting widespread use (Cao and Hewitt, 1991; Koutrakis *et al.*, 1993; also see Appendix 1). The wider the internal diameter and shorter the diffusion length of the tube, the faster the uptake rate and shorter the exposure time. The low uptake rate of such a design is good for relatively long term sampling (8 hours or more). Perkin Elmer tubes (stainless steel, 15mm length x 4.8mm diameter) have an optimised length : diameter ratio to ensure the minimum effect of high face velocity to minimise turbulence within the diffusion air gap of the sampler. This effect is additionally reduced by using a diffusion cap, which also protects from moisture with slight reduction in diffusive path length.

Two essentially different evaluation systems for tube-type diffusive samplers have developed over recent years: 'solid' or 'liquid' adsorptive collection and subsequent laboratory analysis, and the use of reagent layers giving a direct reading. The latter are marked in ppm-hours for the contaminant analysed. The stain length (dose in ppm.hrs) is divided by the exposure hours to determine the concentration in ppm. Such a technique is also useful to determine BTV or cartridge changeover time.

Regarding solid adsorptive sampling, it is essential that the optimum adsorbent 'strength' is selected for a particular analyte in order to keep the analyte concentration at the adsorbent surface as low as possible and the uptake rate constant throughout the whole sampling period (Cao and Hewitt, 1993) (see Appendix 1). The selection process uses either the specific retention volume at 20°C (retention volume of a particular analyte at 20°C per gram of adsorbent, i.e. adsorbent 'strength') or a determination of the experimental uptake rate (Van de Hoed and Halmans, 1987). The retention volume method is the simplest, and must be in excess of 100L/g. The experimental uptake rate should be similar to the ideal uptake rate (Appendix 1) and be constant, or decreasing only very slightly, below certain exposure dose levels (ppm.min).

As each adsorbate will require its own optimal adsorbent, a wide variety of adsorbents have been tested including traditional ones such as Tenax TA®, activated charcoal, Carbotrap® and Chromosorb® (Adkins and Henry, 1993) in addition to more exotic and novel adsorbent systems. Examples of the latter include Tenax-GR® (Tenax matrix filled with 23% graphitized carbon) for isoprene, benzene, toluene etc. and double-layer tube-type systems.

The thermally-desorbable double-layer passive sampler for the sampling of organic vapours uses two different graphitised carbon blacks (CarbopakC® /Carbotrap®) and is particularly useful for samples with a wide range of boiling points. Such multisorbent samplers have been designed based on the findings that medium and high boiling point compounds adsorb primarily at the front of the packing and those with low boiling points at the back (Bertoni *et al.*, 1990). This multisorb sampler was evaluated to determine the influence of wind velocity and direction, sampled species concentration and exposure time on sampling rate, and also desorption efficiencies to ppb level. Laboratory and field tests indicate that small cross-section tubes are independent of these environmental parameters over a wide range of operating conditions. The results were in agreement with those from active sampling of the same tubes.

An example of a tube-type passive sampler using a liquid-type adsorbent, and its development from conception in the 1970's to 1993, is that used in assessing personal exposure to NO_x (Palmes and Tomczyk, 1979). First developed from a NO_2 passive sampler (Palmes *et al.*, 1976) based on the NO_2-TEA (triethanolamine) complexation on stainless steel screens, later modifications using chromic acid impregnated glass fibre filter discs allowed the conversion of atmospheric $NO \rightarrow NO_2$. The total NO concentration was the difference between the two techniques (NO_x-NO_2 = NO). Following the highlighting of the effects that high levels of NO might have on foetal development, a thermal desorption method using a solid adsorbent, cold-trapping and a Poraplot Q gas chromatographic column (Perkin Elmer, 1991b) was developed for rapid automated routine monitoring of hospital anaesthetists etc.

Examples of the application of passive samplers to mountain forest ecosystems include an ozone-fugitive indigo-carmine dye on filter paper, mounted in 25mm diameter polycarbonate holders downwind of PTFE Teflon filters (Grosjean and Williams, 1992). The colour fades in proportion to ozone concentration and is measured using reflectance spectroscopy.

The determination of airborne chlorinated solvents using passive samplers in less remote areas than forest ecosystems has been reported. Gilli *et al.* (1992) used a Zambelli-Milan Italy 'TK-200' passive sampler to assess the concentrations of 1,1,1-

trichloroethane in indoor, outdoor and personal air in parallel with active sampling in urban, rural and remote areas. SKC cartridges (G.A.C. Cat. No. 266-01) were used for the active sampling and analysis for both sampler types was solvent extraction and capillary GC/ECD. The two methods showed good overlap with a correlation coefficient of 0.96. Spherocarb® (activated charcoal) has been used in passive monitoring tubes to determine vinyl chloride monomer concentrations in the workplace using an automated thermal desorption method (Perkin Elmer, 1991c).

Calibration

Several exposure chamber systems have been described in the literature (Bertoni *et al.* 1990; Cao and Hewitt, 1993; Lee *et al.* 1992; Perkin Elmer, 1991a) to enable passive sampler tubes to be calibrated (**Figure 2.2.1.4**) Calibration is defining the collection rate under different sampling rates, air velocities and analyte concentrations. Laboratory chamber tests mimic the combinations of exposure times and concentrations, face velocities (**Figure 2.2.1.5**), relative humidity and temperature that the sampler is likely to encounter during ambient use (Koutrakis, 1993).

Because of the dependence of passive sampler net uptake on the exposure dosage, calibration utilises a relatively high analyte concentration over a relatively short time instead of laborious individual analyte exposure to each adsorbent at ambient concentrations. Such a process could take weeks to complete (Cohen *et al.* 1990), in addition to the difficulties in generating and precisely controlling organic vapours at this 10^{-9}v/v level.

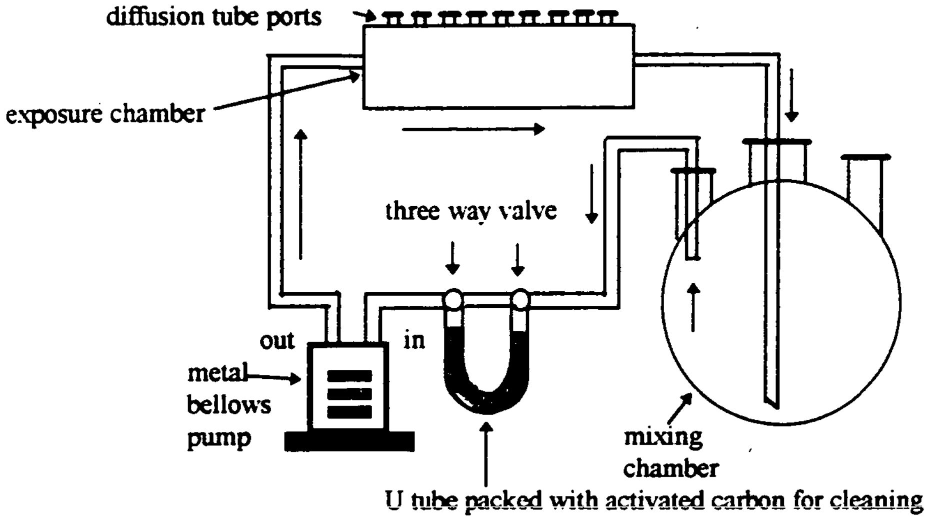

Figure 2.2.1.4 An exposure system for the calibration of passive sampling tubes (Adapted from Cao and Hewitt, 1993). The organic vapour is introduced through an exposure chamber port and circulated by the low capacity metal-bellows pump. Concentrations are determined by GC. The air velocity through the chamber is 79 cm/min. Humidity = 37±3%, Temperature = 21±1°C. Sampler uptake is plotted Vs exposure dose.

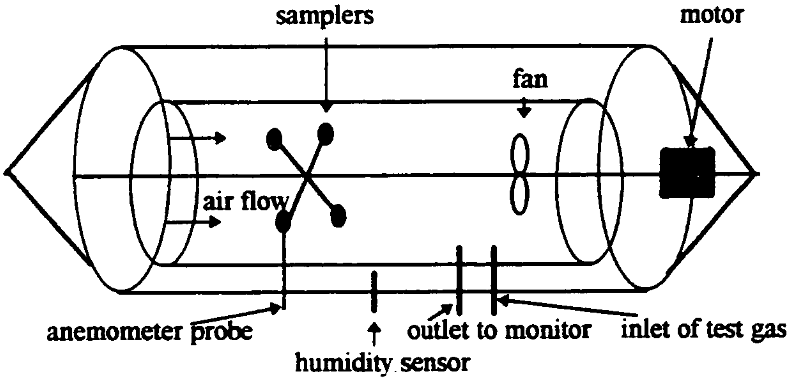

Figure 2.2.1.5 Wind tunnel for the determination of face velocity effects. From Kanno and Yanagisawa (1992). For example, the sampler can be orientated at 0° or 90°to the wind direction to assess wind speed / collection rate relationships.

Sampling strategies and quality control protocols have been established to regulate exposures, primarily in the workplace, by bodies such as the HSE (Brown *et al.*, 1984), NIOSH (Health and Safety, 1987) and the European Committee for Standardisation. The accuracy of determination of chlorinated hydrocarbons in workplace air by personal monitoring techniques using thermal desorption and solvent extraction is under review (Adkins and Henry, 1993).

Uptake rates can be confirmed by passive and active sampler field comparisons. Field calibrations are not practical at extremely low concentrations due to the days/week - long sampling times. Good agreement for the pump and passive systems over an 8 hour sampling time suggests that as long as the adsorbent/adsorbate system is optimised, field calibration at ambient concentrations may not always be essential (Cao and Hewitt, 1993).

Adoption of the passive sampler method

It was decided that the passive sampler technology could be adapted for airborne chlorinated solvent concentration determination at remote mountainous forested sites. As discussed, passive samplers offer several advantages over pump-based, canister and on-line sampling systems:

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(1) robust, lightweight low cost technology;

(2) flexible collection of useful quantitative data of long term mean concentrations with fewer variables to consider i.e. continuous 24 hour profiling rather than `snapshot` or time-weighted average measurements (Perkin Elmer, 1992);

(3) straightforward sampling procedure allowing successful operation by non-technical personnel in remote areas and return of tubes for analysis via the postal service.

(4) no requirement for costly cryogenics and thermodesorption (not available) or power supply at field sites (not available).

The choice of adsorbent

Passive sampler specificity is defined by the adsorbent characteristics. Usually, passive diffusion tubes have only one sorbent per sampler and are not generally used for wide volatility range samples. Low uptake rates mean that there is a lower atmospheric concentration limit (normally > 1ppb).

It was recognised that the uptake rate of volatile species such as chlorocarbons may decline with exposure time to display non-linear adsorption isotherms (**Figure 2.2.1.6**), and are more readily lost by back diffusion. Tenax® is not suitable due to its weak specific capacity, low BTV's for chlorocarbons and tendency towards artefact formation.

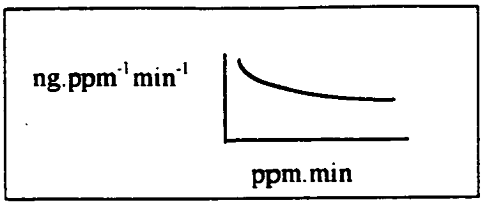

Figure 2.2.1.6 Decline of uptake rate (ng.ppm^{-1}min^{-1})with exposure (ppm.min).

The specific application of the passive samplers was to the large-scale monitoring of chlorinated solvents in air at three forested regions in northern Britain in conjunction with a determination of foliage concentrations. These measurements should allow the partitioning characteristics of these compounds between the ambient atmosphere and leaf wax to be studied. An ideal adsorbent would be one that mimicked the uptake of these compounds in the vapour phase by tree leaves and displayed similar kinetics and enrichment characteristics, without suffering from the low retention volumes, calibration and contamination problems of commercially available adsorbents.

As described in Chapter 1, the plant cuticle is a lipophilic polymer membrane which covers the ariel parts of plants and limits passive and evapotranspirative loss of water to the atmosphere. Volatile organic compounds from the air with high lipophilicity, low solubility in water and high octanol-water partition ratios (K_{ow}) are adsorbed by these leaf surface lipids and epicuticular waxes (Schreiber and Schönherr, 1992) and the attainment of equilibrium is rapid. Such enrichment of chlorinated solvents in the lipids and waxes of cuticles and cellular membranes is due to their large partition ratios (Frank and Frank, 1989).

A Scots pine wax extract was chosen as the sampler adsorbent as it was representative of uptake of chlorinated solvents by whole needles (Chapters 4 and 7). This allowed a simpler chemical system with a high 'capacitance', to display the same uptake and enrichment characteristics as the leaf without interference from biological factors.

Unlike conventional solid adsorbents, the adsorption isotherm is linear until the equilibrium partition ratio is reached between the bulk of the wax and the atmosphere. The analyte concentration at the surface of the wax adsorbent slowly increases through the exposure time as molecules move through the wax from the surface, and air:wax equilibrium is attained rather than surface saturation with adsorbed analytes.

In addition, such devices are self-correcting being exposed simultaneously with the tree leaves to changes in environmental conditions. Sampler construction is simplified as the sampling wax adheres to the sampler tube, directly removing the need for containing gauzes for the adsorbent (Cao and Hewitt, 1991).

2.2.2 Passive air sampler construction and validation

The design goals during sampler construction were that;

(1) As discussed in Section 2.2.1, a wax extract should be used as the optimal adsorption medium of such a thickness to provide sufficient capacity as dictated by analytical sensitivity, and simulate chlorinated solvent movement from the surface to interior with retention characteristics analogous to those of the real leaf wax.

(2) A long and narrow diffusion tube (small cross-sectional area) should be used to maximise the area : length ratio and provide a long diffusion path length to minimise turbulence effects, i.e. stable uptake rate over a wide range of ambient wind speeds;

(3) The wax adsorbent should be shielded from light due to the low photostability of chlorocarbons;

(4) A suitable cap must be incorporated for sealing, to promote stability over time and storage prior to exposure and before analysis;

(5) Either thermal desorption or solvent extraction techniques can be used;

(6) It should be simple to use in the field, lightweight and resilient enough for long exposure periods and dependence on the postal service for transportation;

(7) All traces of contaminants are able to be removed from the sampler tube walls by normal cleaning procedures, and no organopolymers are present.

The sampler design adopted is shown in **Figure 2.2.2.1**.

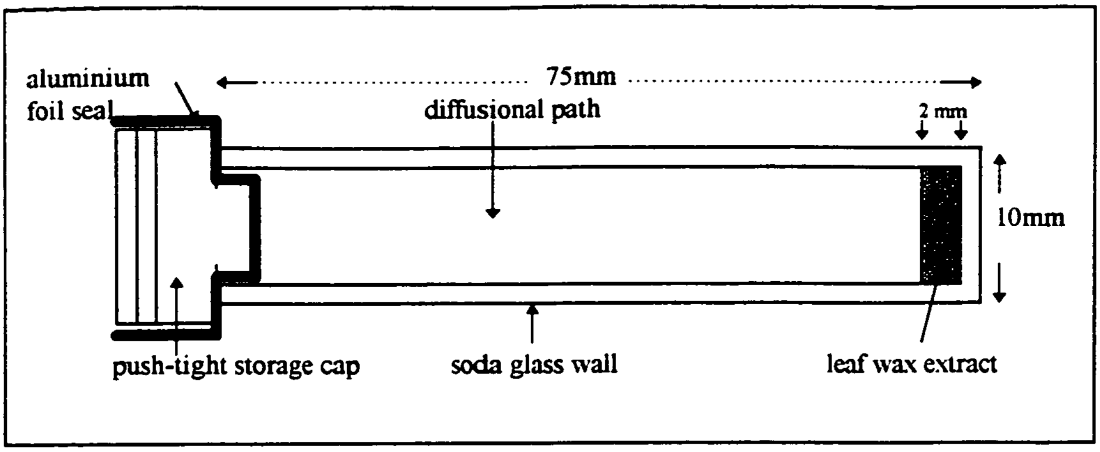

Figure 2.2.2.1 Passive air sampler design for chlorocarbon sampling in remote forest locations. Tube OD = 10.47 mm, ID = 9.47 mm, length = 75mm (Regina Industries Ltd., UK).

Two methods were investigated to determine the most appropriate choice of wax adsorption medium for use in the tube-type samplers. A comparison of the results of blank level determination and ambient air exposure trials of the wax adsorbent produced by the two methods led to adoption of leaf wax extract as the adsorbent.

(1) Paraffin wax method

Paraffin wax was purified by heating of impure wax (Aldrich, mp = 56-61°C) to 70°C (oil bath) under vacuum for 4 hours while stirring with a glass-coated magnetic bead (**Figure 2.2.2.2**).

Solutions of 1mg/ml impure and pure wax (mp = 58-60°C) in purified hexane were made up to represent the approximate concentration of the leaf wax sink based on a 1% lipid content per needle (100 - 200 µg / needle dry weight). It was found that such solutions did not solidify at ambient temperatures greater than 18°C, although cooling to 11°C promoted precipitation of wax on sampler tube base. Solutions of the purified wax at higher concentrations (15 mg/ml and 35 mg/ml) were made up, but the wax melting point was still depressed by the hexane impurity and no solidification was observed.

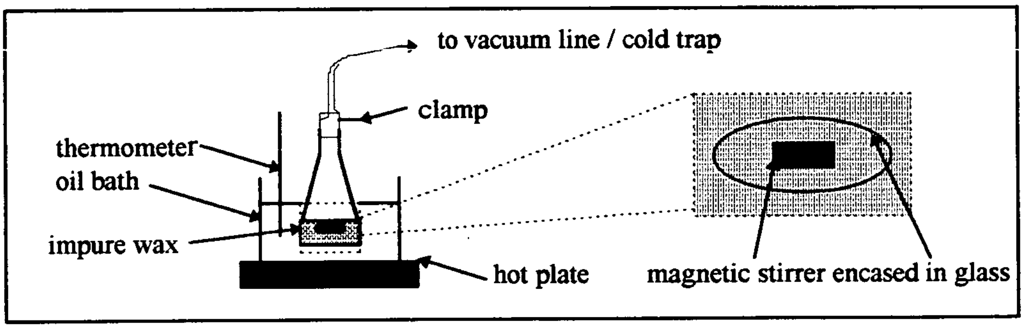

Figure 2.2.2.2 The purification of paraffin wax

(2) Leaf wax extract method

Approximately 20g (wet weight) of Scots pine (*Pinus sylvestris* (L.)) needles (1992 year class) were scissor cut from a stand of trees 20 - 30 years old at King's Buildings, Edinburgh, directly into a pre-weighed Soxhlet thimble. The thimble was re-weighed and transferred to a standard continuous Soxhlet extractor in a fume hood (**Figure 2.2.2.3**). 200 ml of HPLC hexane (Rathburn) was used as a cost effective extraction solvent of suitable purity and boiling point (69°C) over a period of 6 hours at a temperature of 70°C.

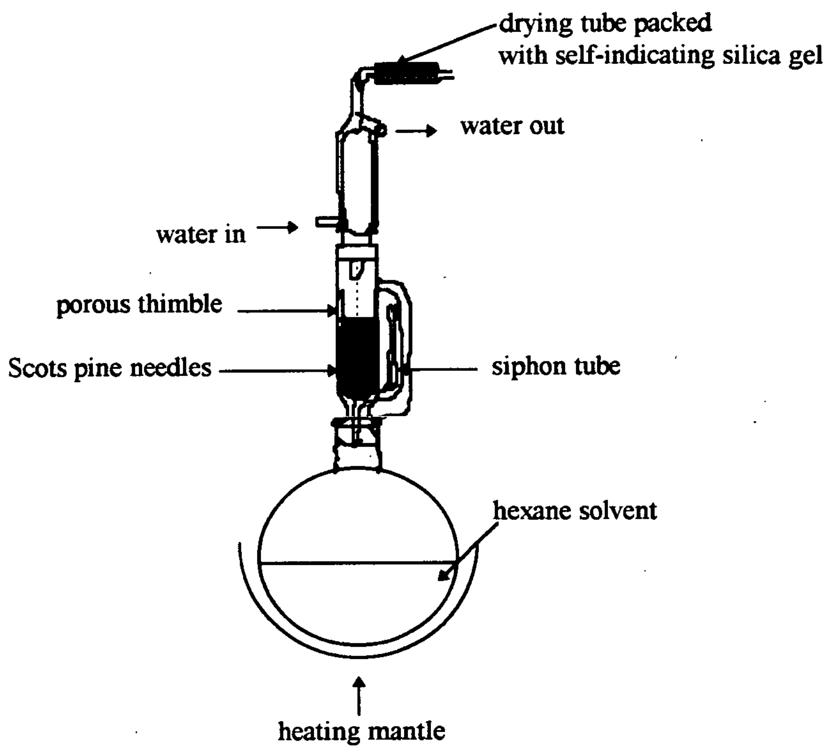

Figure 2.2.2.3 Continuous Soxhlet extraction of Scots pine needles.

After cooling, the flask containing the extraction solvent was stored for 24 hours at 4°C to allow wax flocculation, before rotary evaporation at 40°C (to minimise wax decomposition) to reduce to a volume of 20ml. A 2ml aliquot of this extract was syringed under fume-hood conditions into pre-cleaned sampler tubes and the tubes were placed in an oven at 55°C. Excess solvent evaporated leaving a 2mm film of the leaf wax extract coating the inside base of the sampler tube. The tubes were immediately capped and stored at room temperature prior to use as blanks or exposure in the field.

It was necessary to know the mass of wax in each sample tube. Wax density and film height were used, the number of sample tubes to be made up precluding mass by weight difference measurements. Wax density was also required for partition ratio studies (Chapter 4). From these measurements the mass of wax present per tube was found to be 0.12 ± 0.01g.

Molten wax extracted as above with complete removal of solvent during rotary evaporation was drawn up into a pre-weighed capillary tube (n=11). The wax was allowed to solidify and then the length and weight of the resultant wax 'column' determined. Thus the density can be calculated as 0.82 ± 0.06 g/cm^3, the diameter of the capillary being known (0.94 mm). The measurements and calculations made are shown in Appendix 2.

Care must be exercised by appropriate control of the glass transfer syringe to ensure the wax does not creep up the insides of the tubes, as this would reduce the effective diffusion path length. Over-heating in the oven whilst driving off excess extraction solvent can also have this effect.

In both the paraffin wax and leaf wax extract methods, all experimental glassware and glass syringes were cleaned rigorously using a detergent wash followed by a rinse of de-ionised water. In addition, passive sampler tubes were washed with a chromic acid solution prior to the detergent wash. The glassware, syringes and tubes were oven dried for 24 hours at 110°C before use.

Method comparison

The leaf wax method was adopted as providing the superior adsorbent for the following reasons :

(1) Optimised solvent extraction with gas chromatographic analysis, (Chapter 3) to determine chlorocarbon concentrations (n = 10), showed that the highest residual blank levels (except for CCl_4) were in the impure paraffin wax adsorbent (**Table 2.2.2.1**). The chlorocarbon levels found in the leaf wax extract were the lowest, with about a 50% reduction over the purified paraffin wax for CCl_4 and C_2HCl_3. The levels able to be determined in the leaf wax extract were actually limited by the blank levels found in purified hexane (Chapter 5).

Table 2.2.2.1 Optimised gas chromatography blank level determinations for purified hexane extractant and passive air sampler tubes made with impure and purified paraffin and leaf-wax extract adsorbents. The desorption method is extraction in 1 ml purified hexane for 24 hours at 4°C. All results are given in pg per injection and expressed as the mean ± standard deviation (n=10).

Chlorinated solvent	purified hexane	impure wax 1 mg/ml	purified wax 1 mg/ml	leaf wax 1 mg/ml
$CHCl_3$	1.3±0.77	1.7±0.01	1.6±0.35	<1.6
$C_2H_3Cl_3$	1.7±1.5	6.1±4.2	3.2±0.22	2.7±0.52
CCl_4	0.52±0.32	0.89±0.71	2.8±0.21	1.4±0.89
C_2HCl_3	6.7±3.7	21 ±13	21 ±14	<12
C_2Cl_4	2.5±1.2	<2.5	<2.5	<2.5

(2) The low melting point of the paraffin wax when transferred by the preferred method of a solution in hexane is a problem, as ambient temperatures are often greater than 18°C. Other techniques investigated included grating of the wax into the tube and melting to form a disc, or transfer of a bored solid disc to the tube. Both

increase exposure to chlorocarbon-laden laboratory air. The rotary evaporation and oven-drying processes during the production of the leaf wax extract samplers drives off volatile contaminants, including chlorocarbons, significantly reducing blank levels.

(3) Trial results from both types of air sampler exposed for a 20 day period to outdoor air at the King's Buildings site show that the leaf wax extract is a more suitable adsorbent (**Table 2.2.2.2**).

(4) Following from Section 2.2.1, the leaf wax extract is much better as an analogous system to the real leaf wax, reflecting the natural properties of the surface which promote uptake and accumulation of chlorinated solvents. The air sampler calibration and validation procedures required are significantly reduced.

(5) Ultrapure pentane (low haloform, Rathburn) was adopted as the extraction solvent for passive air sampler analyses (Chapter 3).

Table 2.2.2.2 GC/ECD results in pg per g from purified paraffin wax and leaf wax air samplers exposed for a 20 day period to outdoor chlorocarbon concentrations at King's Buildings during November 1992 (mean air temperature = 6°C). Samplers were desorbed in 1 ml purified hexane for 24 hours at 4°C before analysis under optimised GC/ECD conditions (Chapter 3). Values are mean ± standard deviation. The detection limit is twice the standard deviation of the blank mean.

Chlorinated solvent	Detection limit (dl)	Adsorbent	
		Purified paraffin wax	Leaf wax extract
$CHCl_3$	3.2	<dl	4.5±3.4
$C_2H_3Cl_3$	9.8	<dl	13.33±4.8
CCl_4	2.5	<dl	<dl
C_2HCl_3	37	<dl	<dl
C_2Cl_4	13	<dl	<dl

Validation of passive air samplers

An initial study at Ponsonby Tarn forest for 1 month (**Table 2.2.2.3**), and replication studies of leaf wax extract air sampler tubes exposed in the field as given in **Table 2.2.2.(4,5)** confirm that leaf wax can be used as an adsorbent to measure some chlorinated solvents from those of interest. Chapter 3 gives details of experiments involving the spiking of the sampler adsorbent with known amounts of chlorocarbons. These data confirm that GC/ECD methodology and retention time assignments are correct.

Table 2.2.2.3 Initial field study at Ponsonby Tarn forest to determine suitability of leaf extract air samplers exposed for 1 month (01/03/93 to 01/04/93) and then analysed using solvent desorption and GC-ECD. Results are given in pg/injection, expressed as mean ± standard deviation, n=3.

Chlorinated solvent	km from BNF plc. Sellafield		
	0.5	1.0	1.5
$CHCl_3$	<53	6360±5900	<3670
$C_2H_3Cl_3$	<19	<19	<19
CCl_4	<4	22±13	<4
C_2HCl_3	<140	<140	<140
C_2Cl_4	<12	8±3	12±8

Table 2.2.2.4 Replication studies from leaf wax extract passive air samplers exposed for 1 month from 12/02/93 at Devilla forest. GC-ECD analysis in triplicate on 6 exposed sample tubes, 1-3 and 4-6, one set on each of two supports at 13m height (top of canopy, Section 2.3.1). Results are given in pg/injection, expressed as mean ± standard deviation. n=3. Detection limit (dl) is twice standard deviation of the blank mean.

	dl	Passive air sampler tube						Reported as
		1	2	3	4	5	6	
$CHCl_3$	6200	<dl	<dl	<dl	<dl	<dl	<dl	<dl
$C_2H_3Cl_3$	20	<dl	<20	<dl	<dl	<dl	<dl	<dl
CCl_4	1.2	1.9±1.2	1.6±0.9	<dl	1.6±0.9	1.7±1.4	1.2±1.0	1.7±1.0
C_2HCl_3	15	<dl	<dl	<dl	<dl	<dl	<dl	<dl
C_2Cl_4	11	<dl	<dl	<dl	<dl	<dl	<dl	<dl

Table 2.2.2.5 Replication studies for 9 air samplers exposed for one month from 22/02/94 at 1.8m height at Banchory forest. GC/ECD analysis in triplicate, results in pg/injection (mean ± standard deviation, n=3). Detection limit (dl) is twice the standard deviation on the blank mean.

Chlorinated solvent	dl	Air sampler 1	2	3	4	5	6	7	8	9
$CHCl_3$	420	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
$C_2H_3Cl_3$	2	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
CCl_4	1	1±1	<dl	4±2	1±1	3±2	<dl	1±1	3±1	<dl
C_2HCl_3	6	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
C_2Cl_4	28	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl

Conclusions

The preliminary results shown in Tables 2.2.2.(3-5) show that the GC/ECD data obtained is close to the detection limit. CCl_4 and C_2Cl_4 are most likely to be detected. The large blank values for $CHCl_3$ are discussed in Chapter 5 as is the probability plot method for limit of detection calculations.

2.2.3 Choice and location of sampling sites

2.2.3.1 General considerations

The target forests were chosen based on the project objectives listed in Chapter 1.

The selection criteria for forest choice were, in each forest:

(i) A stand of Scots pine (*Pinus sylvestris* L.) as the target species had to be available for monitoring of both air and foliage over a 15 month period (March 1993 to June 1994). The stands in each forest had to be of similar aspect, elevation, age, tree density and size;

(ii) An area of 100 m^2 had to be available for spatial distribution of sampling ;

(iii) Vehicle access for personnel was needed;

(iv) A near-site building for storage of passive sampler blanks was available;

(v) Foliage sampling for at least 2 needle class years could be designed around fixed air monitoring sites ;

(vi) Wind speed/direction data for the forests during the 15 month sampling programme could be obtained.

A suitable forest (Ponsonby Tarn) was chosen near British Nuclear Fuels plc., Sellafield, West Cumbria in accord with CASE award status. The proximity of Edinburgh University to petrochemical industry at Grangemouth in Central Scotland made Devilla forest a logical choice for a second ‘industrial’ forest in conjunction with the Institute of Terrestrial Ecology (ITE) research station at the Bush Estate, Edinburgh. A third forest, near ITE Brathens, at Banchory in north east Scotland was adopted to represent a ‘remote’ location.

2.2.3.2 Site description

Ponsonby Tarn forest

Ponsonby Tarn forest is situated in West Cumbria 16 km south east of Whitehaven and 6 km from the coast of the Irish Sea (O.S. Grid Reference 047045). The experimental section of the forest is 55 - 80 m above sea level and consists primarily of Norway spruce (*Picea abies* (L.)) and European larch (*Larix decidua* L.) interspersed with blocks of Scots pine (*Pinus sylvestris* (L.)). The sites used in this study are reasonably sheltered from the (generally) SW winds in the centre of 0.2-0.4 ha blocks of Scots pine. The western forest boundary is 0.5 km from the Sellafield perimeter fence. Access is via a farm road leading from the A595 to the east of the forest.

Devilla forest

Devilla forest is situated in central Scotland, 30 km NW of Edinburgh (O.S. Grid Reference 958894) 12 km due north of the Grangemouth petrochemical complex across the River Forth estuary (**Figure 2.2.3.2.1**). The forest is 75m above sea level and has a southerly aspect. The forest is predominantly of Scots pine, although a few smaller blocks of European larch are present. Access is by farm / Forest Enterprise tracks leading from the A985(T) south of the forest.

Banchory forest

Banchory forest is situated in NE Scotland approximately 30 km WSW of Aberdeen (OS Grid Reference 683982). The forest lies at 110m above sea level on the north side of the River Dee and has a southerly aspect. The experimental area uses a block (0.6 ha) of Scots pine in a mixed forest containing Sitka spruce (*Picea sitchensis*) and Beech (*Fagus sylvatica*). Access is by a minor road off the A950 to the south of the forest.

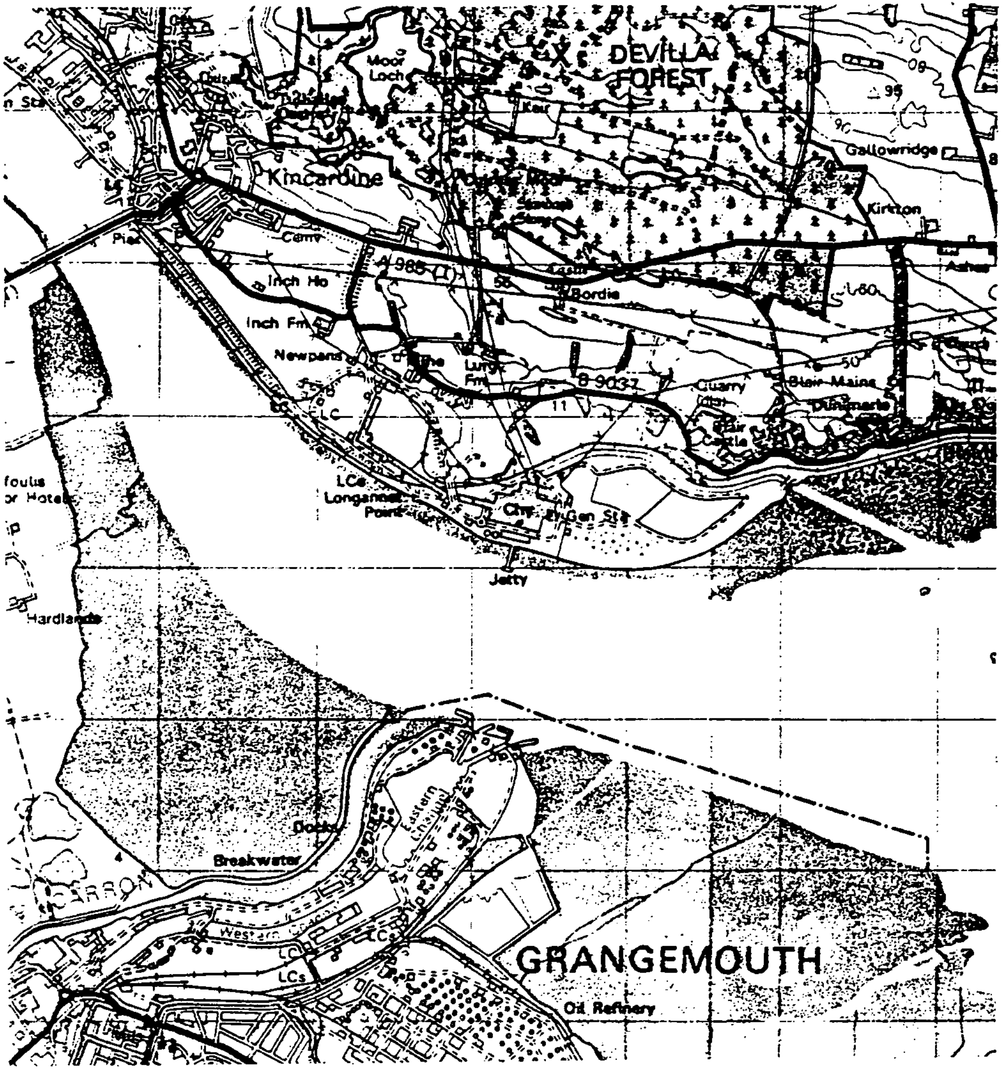

Figure 2.2.3.2.1 Location of Devilla forest due north across the River Forth estuary from petrochemical industry at Grangemouth. The sampling site is marked (**x**).

2.2.3.3 Site specific considerations

Table 2.2.3.3.1 provides some site-specific information on the Scots pine stand properties at each of the three forest locations.

Table 2.2.3.3.1 Scots pine stand properties for the three target forests.

Site	Planting Date	Stem Density (ha^{-1})	Tree Height (m)
Ponsonby Tarn	1955[a]	3000	15
Devilla[b]	1952	3900	13
Banchory[b]	1959	2700[c]	16

[a] estimated date of planting, [b] from Cape *et al.* (1991),[c] thinned since planting

Sampling sites at 0.5, 1.0 and 1.0 km from the Sellafield boundary were designed to consider a lateral transect across the forest from the factory (**Figure 2.2.3.3.(1,2)**). Air sampling was uniform at 1.8m from the ground to emulate the real leaf situation whilst being accessible from the ground. Six air sampler tubes in two sets of three were attached at each sample site, one to each of two trees 5 m apart. **Figure 2.2.3.3.3** shows an air sampler set in position in Ponsonby Tarn forest.

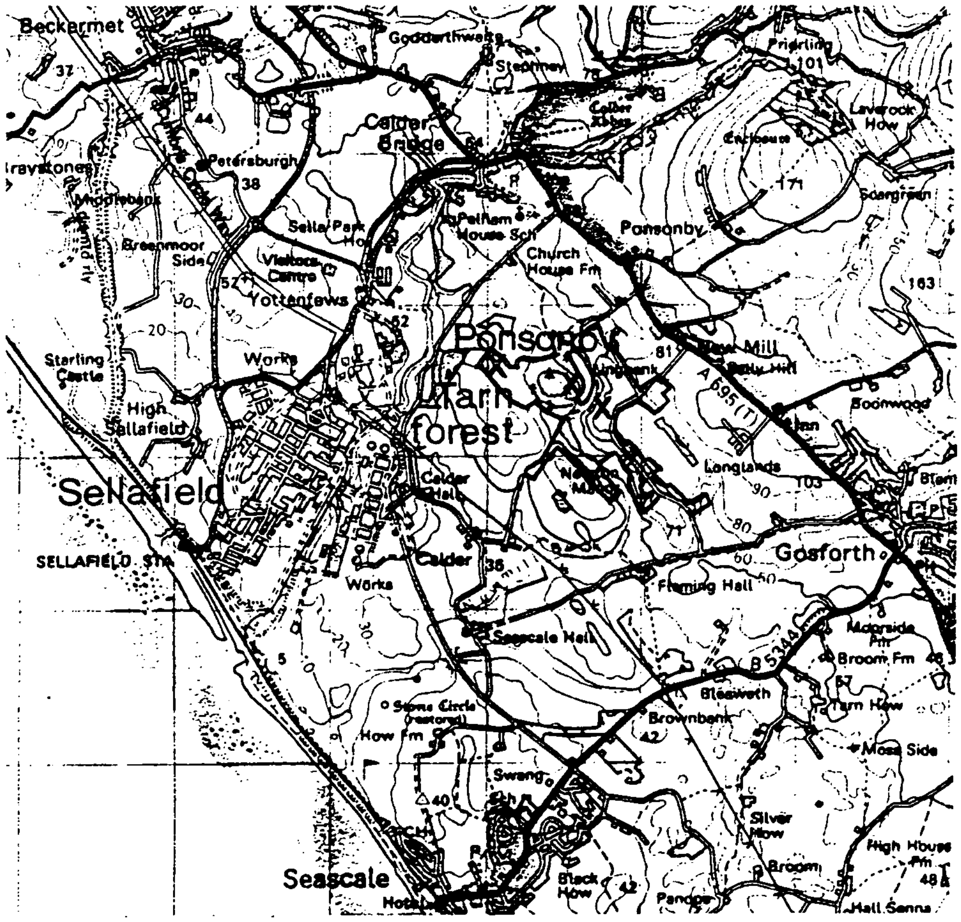

Figure 2.2.3.3.1. Map showing the sampling transect across Ponsonby Tarn forest away from the potential area source of BNFplc. Sellafield, **x** = sample sites.

Figure 2.2.3.3.2 Photograph showing Ponsonby Tarn forest to the east (left in the photograph) of the BNFplc. Sellafield site (courtesy of A. Brown).

Figure 2.2.3.3.3 Photograph of an air sampler set in position in a Scots pine tree at the most westerly sampling site, Ponsonby Tarn forest (courtesy of A. Brown).

A holder provided mechanical support and protect the adsorbent from sunlight (**Figure 2.2.3.3.4**), wax integrity was maintained and photoreaction of accumulated chlorinated solvents prevented.

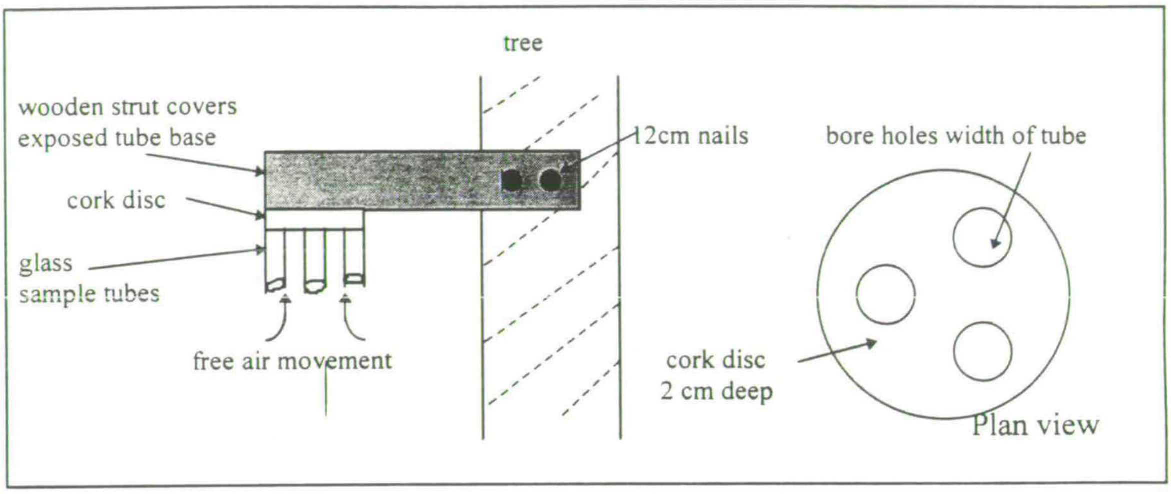

Figure 2.2.3.3.4 Air sampler holder design and attachment.

The gale of 03/02/94 blew down one of the sampling trees at the most westerly Ponsonby Tarn site. An adjacent tree was adopted as a replacement for the remainder of the sampling programme, until 23/06/94.

Devilla forest

Air sampler holders were attached by clamps to the struts of a fixed mast allowing vertical profiling of the air chlorocarbon burden. This allowed consideration of plumes resulting from Grangemouth emissions. The passive sampler tubes were mounted (**Figure 2.2.3.3.5**) at 7m (base of leaf canopy), 10m (mid-canopy) and 13m (top of canopy). Two sets of three tubes in holders were mounted at each height.

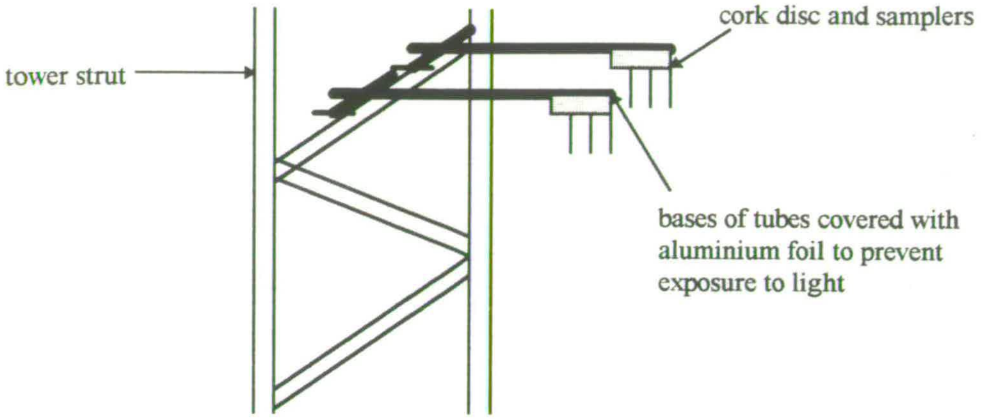

Figure 2.2.3.3.5 Clamping passive air sampler holders with tubes inserted to the struts of the fixed mast in Devilla forest. Repeated at 7m, 10m and 13m heights.

Banchory forest

A triangular arrangement was adopted for the air samplers at this forest site to provide a large enough area for spatial sampling distribution (**Figure 2.2.3.3.6**). Air samplers were again at a uniform 1.8m from the ground and attached to trees as at Ponsonby forest.

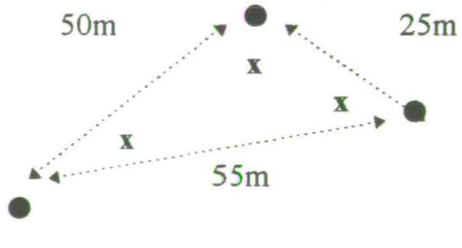

Figure 2.2.3.3.6 Triangular distribution of air sample positions at the Banchory forest site. Each air sampling position supports 6 passive sampling tubes in two sets of three (as Figure 2.2.3.3.4). Both sets were mounted on the same tree at each sampling position, **x** = approximate location of foliage sampling positions.

2.2.4 Storage of air samplers and sampling frequency

Storage of air samplers

Prior to exposure in the field, or blank field storage, air samplers were wrapped in aluminium foil and stored in card boxes, in fume hoods at room temperature. At each forest location, the non-exposed blanks are left, labelled, sealed and wrapped in aluminium foil in a card box, in either an on-site hut (Devilla), outhouse (Ponsonby) or temporary office (Banchory) for the duration of the equivalent exposure period.

Following exposure, the air samplers are returned to the Department of Chemistry and stored, again wrapped in aluminium foil, at 4°C for up to 3 months prior to GC/ECD analysis. No loss of chlorinated solvents or observable wax decomposition occurs during this period (**Table 2.2.4.1**). The unexposed blank air samplers undergo identical treatment following return from the field.

Table 2.2.4.1 Reproducibility over time of air samplers exposed 06-10-93 to 23-11-93 at the 13m Devilla site (n=3). GC-ECD analyses are in triplicate. Results are in pg/injection and expressed as the mean ± standard deviation. Samples left for 4 months at 4°C show an insignificant change in chlorinated solvent content.

Chlorinated solvent	Analysis date		
	24/11/93	21/01/94	18/03/94
$CHCl_3$	380±240	370±300	260±210
$C_2H_3Cl_3$	<7†	<10	<5
CCl_4	2±1	3±2	2±1
C_2HCl_3	40±30	50±1	47±27
C_2Cl_4	<4	<3	<6

†The < values vary over time reflecting the blank values (refer to Chapter 5)

Sampling frequency

The initial passive sampling programme focused on monthly air sampling at each forest location, later adapted to be at approximately two monthly intervals in line with foliage sampling considerations.

Although most of the sampling was done by myself, a number of willing volunteers assisted during the monitoring programme with foliage sampling and by installing, collecting and returning tubes to Edinburgh University for analysis. Tubes were returned either in person or via the postal service.

2.3 Foliage sampling method

2.3.1 Choice and location of sampling sites

The siting of the sampling positions for Scots pine needles reflected the siting of the passive air samplers at each target forest.

The site specific considerations were :

Ponsonby Tarn forest

Two suitable trees 5m apart and with good foliage cover were identified at each of the three lateral transect sampling sites (0.5, 1.0 and 1.5 km from Sellafield). One air sampler set was attached to the trunk of each tree as Figure 2.2.3.3.4. Three branches from each of the two trees were collected as in Section 2.3.2 from mid-canopy height (10m). A composite sub-sample from the six branches was scissor-cut into the extraction vial, storage flask or vial for freezing. Six further trees, two at each sample site, were individually harvested for the representativity study (Section 2.3.4).

Devilla forest

The air sampling procedure used the fixed mast for sampler attachment instead of the tree trunk (Figure 2.2.3.3.5). Trees were chosen for needle sampling on the edge of the stand to allow sampling access via a grassy ride. Stand density was too great for sampling at the centre. Although vertical air profiling was at three heights representing top (13m), mid (10m) and base (7m) of the canopy, only the two highest positions could be sampled for foliage - the canopy being too sparse at 7m. Three trees 10m apart were chosen as suitable for sampling, three branches at each height were removed and a composite sample from all nine branches field extracted or stored as before. Nine additional trees at 5m intervals along the ride were individually sampled for the representativity study.

Banchory forest

At the sample site in this forest, the three foliage sampling positions were located as in Figure 2.2.3.3.6 close to the air sampling positions. One tree at each position was identified, three branches from each being removed at mid-canopy (10m) height and composite sub-samples of the nine branches extracted or stored as before. Nine additional trees within the triangular sampling site were selected at random and individually sampled during the representativity study.

2.3.2 Sampling technique and frequency

A squirrel pruner was used to sample the tree foliage from heights up to 15m (Cape *et al.*, 1991).

Foliage samples from each forest location were collected at two-monthly intervals from March 1992 to June 1993. This provided a suitable temporal scale throughout the duration of the sampling programme, consistent with the growing season of the trees, and within the geographical constraints resulting from location of forests.

Three or more easily identifiable needle year classes can generally be collected per sampling. Only 1992 year class needles, however, were collected until the new growth in spring 1993, which then allowed two year classes to be sampled from August 1993 onwards. The actual growth of a needle is complete by July-August of its year of emergence (Chapter 8).

Replicates were taken as 'backups' as a matter of course to guard against sample loss and analytical interference or downtime.

2.3.3 Previous analytical methods

Dedicated chlorinated solvent field experiments, exposure chamber analyses, partitioning studies and the determination of fugacity† measurements all require reliable and reproducible determination of chlorocarbons in the lipophilic regions of plant tissues (Diezel *et al.*, 1988).

The techniques considered in this section primarily focus on extraction from coniferous needle wax/cuticle. The extraction of higher molecular weight chlorinated species dominates the literature (Granier and Chevreuil, 1992). Five principal method types for extracting enriched chlorocarbons from foliage can be identified, although there is considerable overlap regarding sample preparation and cleanup techniques :

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† The fugacity concept describes the environmental partitioning of organic substances as the tendency of a compound to escape to a particular compartment related to chemical potential. If this tendency is equal in two adjoining phases, an equilibrium exists, but differences will result in transport by diffusion of a contaminant to the phase with lower fugacity at a rate proportional to the fugacity difference. Thus, fugacities are normalised concentrations that can be directly compared amongst different matrices, and can be used to evaluate the degree of contamination and predict diffusive fluxes (Horstman and McLachlan, 1992).

(1) **Solvent extraction** followed by capillary GC-MS or GC/ECD (Jensen *et al.*, 1992; Reischl *et al.*, 1988) analysis.

Examples include use of ultrapure *n*-hexane to extract chlorocarbons from intact spruce needles (Frank and Frank, 1989) and dichloromethane, or dichloromethane/ pentane (9:1), to desorb DDT, PCB's, HCH's (lindane), pentachlorophenol and hexachlorobenzene (Reischl *et al.*, 1987; Strachan *et al.*, 1994) from pine, spruce and other foliage (Eriksson *et al.*, 1989; Simonich and Hites, 1994). Sonication (Simonich and Hites, 1994) or homogenisation (Bacci *et al.*, 1990) are techniques sometimes used prior to extraction with solvents such as *n*-hexane/acetone; wax particulates can be removed by filtration or centrifugation. A method requiring grinding of the foliage with an Ultra-Turrax homogeniser followed by ultrasound extraction in *n*-hexane and a Florisil® column cleanup has been described, again for semi-volatile organic pollutant (SOP) determination (Reischl *et al.*, 1987).

Similar techniques have been applied to the determination of SOP's in lichens (Muir *et al.*, 1993). Samples can be dried before extraction in dichloromethane/hexane, separated using a Florisil® column, and analysed using GC-ECD. Lichens are useful atmospheric monitoring tools due to their dependence on the atmosphere for nutrients rather than the substratum and wide distribution - some species of lichen can be seen as 'carpet' species. For the biomonitoring of some atmospheric components, the lack of cuticle and internal transport mechanisms are advantageous.

(2) **Mulching, or milling**, of the foliage in the presence of an extracting agent, such as ethyleneglycolmonomethylether, followed by ultrasound and vacuum condensation, separates aqueous phase extract from spruce needle. An enrichment step such as extraction into toluene from the aqueous phase allows concentration determination under optimised GC-ECD conditions (Diezel *et al.*, 1988).

(3) **Soxhlet apparatus** has primarily been used for the extraction of the semi-volatile non-polar, low degradability organic pollutants (vapour pressure less than 1 kPa at 20°C) known as SOP's taken up from the vapour phase by foliage. SOP's include chlorinated pesticides, polychlorinated biphenyls (PCB's), substituted phenols and polycyclic aromatic hydrocarbons (PAH's)[†] (Simonich and Hites, 1994). These compounds are persistent 'fingerprints' characteristic of a particular area dependent on history of use and socio-economic patterns (Calamari *et al.*, 1994; Chemistry and Industry, 1994). As such, SOPs in matrices such as pine needles act as biomonitors and are 'handles' for equally wide-spread but less easy to detect atmospheric pollutants (Simonich and Hites, 1994).

Many studies look at the partitioning process between atmosphere and the surface of the earth as an ambient temperature - dependent phenomenon (Simonich and Hites, 1994). It has been shown that lipophilic species transported in the atmosphere accumulate in the wax surfaces of sugar maple leaves and the needles of pine trees

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[†] DDT = 1,1-bis (4-chlorophenyl)-2,2,2-trichloroethene
DDE = 1,1,-bis (4-chlorophenyl)-2,2,dichloroethene
PCB's = chlorine-substituted biphenyls
HCH's = hexachlorocyclohexanes (α, γ isomers)

(Hites and Simonich, 1994). This shows the potential of plants to scrub compounds from the atmosphere (Simonich and Hites, 1994).

Soxhlet extraction often follows sample drying and homogenisation (Calmari *et al.*, 1994; Gaggi and Bacci, 1985; Morosini *et al.*, 1993). The extraction solvent used is generally *n*-hexane over a 6-8 hour period. Reduction of the extractant volume is generally by rotary evaporation at a low temperature (45°C) ; sulphuric acid cleanup and Florisil® column chromatography (*n*-hexane elutant) and/or silica gel column chromatography before capillary GC/ECD of the elutant are used. Analysis is by GC/ECD (Calmari *et al.*, 1991), sometimes with GC/MS confirmation (Gaggi *et al.*, 1985).

Uptake rates of chlorocarbons and SOP's into needles have been investigated using radioactive isotopes as tracers, e.g. ^{14}C, with appropriate detection devices such as liquid scintillation counting (Schreiber and Schönherr, 1993). Examples include the adsorption /absorption enrichment of trichloroethene in spruce needles (Figge, 1988) and attempts to determine the leaf compartments where organics are located by looking at whether the radioactivity is sorbed superficially or taken up irreversibly into the needle interior (Schreiber and Schönherr, 1992).

(4) **Headspace** extraction followed by capillary GC-ECD has been used to measure chlorocarbon concentrations in foliage (Herman *et al.*, 1992; Plümacher and Renner, 1991; Plümacher and Renner, 1993). The phytotoxic oxidation product of C_1/C_2 chlorocarbons, trichloroacetic acid (Frank *et al.*, 1994) can also be determined by exploiting the observed thermal decarboxylation to carbon dioxide and chloroform

using headspace GC, or deriving it as a methyl ester for capillary GC/ECD analysis (Frank *et al.*, 1992).

(5) The examples of **thermal desorption** methods, which dominate the literature when considering the sampling of air for chlorinated solvents, are not as prominent when considering foliage. High temperature thermal stripping with thermal desorption - gas chromatography has been suggested as a quick effective method with good reproducibility for determining non-volatile organics in environmental samples (Brown *et al.*, 1991). Carbotrap 150 tubes are used as the adsorbent, the thermal stripping unit (**Figure 2.3.3.1**) is used for foliage sample preparation by steam distillation and the thermal desorption unit is interfaced to capillary GC/ECD.

In this project, a thermal desorption method was adapted to desorb chlorinated solvents from both air sampler leaf wax extract and foliage, i.e. reversing the process whereby the chlorocarbons entered the adsorbent. The passive sampler or leaf sample was heated using a water bath at 50°C. The released volatiles were cold trapped (-177°C liquid N_2) and transferred directly to the GC capillary column by rapid heating to 100°C (boiling water) through a stainless steel coil interfaced to the GC injection port via the split vent (Section 2.3.4).

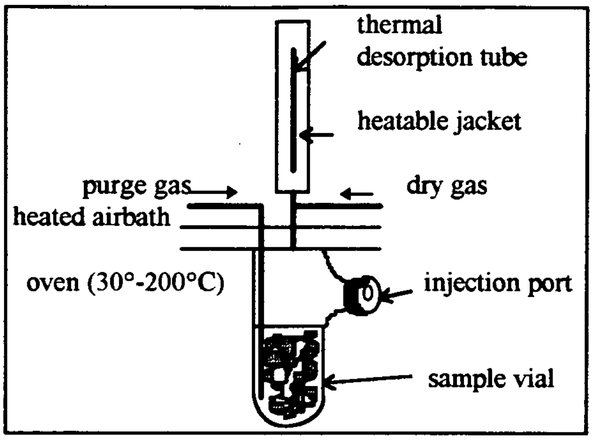


Figure 2.3.3.1 High temperature thermal stripping device. The organics adsorbed in the tube are thermally desorbed and analysed by GC/ECD (from Supelco, 1991).

Major problems can be experienced when using any of these methods due to the presence of several ubiquitous halocarbons in laboratory air at relatively high levels. Sample preparation must be performed in a clean fumehood equipped with an air purification system filled with activated charcoal (Frank and Frank, 1989), or preferably carried out at the field site.

In any method involving solvents, the purification of such chemicals must be rigorous to reduce any halocarbon contaminants to acceptably low levels for the analytical procedure used. Chapter 3 details the steps used to purify hexane and pentane, e.g. refluxing over dry sodium and distillation or glass distilling and filtering through aluminium oxide (Strachan *et al.*, 1994). Toluene can be distilled over Na - Pb and stored in glass-stoppered flasks for several days. Ethyleneglycolmonomethylether is distilled over dried Na shortly before use but stays free of chlorocarbons for no longer than 3 - 4 hours. Water extracted into 10% cleaned toluene using ultrasound will remain free of chlorocarbons for 2 - 3 days under the toluene (Diezel *et al.*, 1988).

All sample vials and glassware must be rigorously cleaned as appropriate and oven dried at >100°C. Examples of cleaning routines include ethanol and acetone (Strachan *et al.*, 1994), toluene rinse (Diezel *et al.*, 1988), detergent at 40°C in a circulating bath / 24 hour 5% nitric acid soak / water rinse (Allchin *et al.*, 1989) and chromic acid, detergent and de-ionised water washes (Brown *et al.*, 1995). It must be ensured that cleaned, dry sample vials are closed securely prior to use.

Analytical technique detection limits

As the median air concentration in rural areas of the chlorinated solvent of interest is $\sim 1 \mu g/m^3$, detection down to 100 pg/g of needle by the analytical technique of choice is required based on partition ratios determined in model experiments (Frank and Frank, 1986; Chapter 4,6). Detection limits of 1 ($C_2H_3Cl_3$) - 3 (C_2Cl_4/$CHCl_3$) ng/g spruce needles using the milling extraction technique and GC-ECD have been reported (Diezel *et al.*, 1988).

Compounds within samples with electrophores such as aromatic aldehydes, or quinones, may interfere in the electron capture detection (Vessmann, 1980). Frank and Frank (1989) report lower detection limits (~ 0.3 pg ± 40% for $C_2Cl_4 \equiv 0.2$ ng/cm^2 needle $\approx$ 100 pg/g spruce needle with a signal to noise ratio of 3) when using chemical ionisation mass spectroscopy monitoring the negative chlorine ions at m/z 35 and 37. The noise level varies within 40% from day to day as the ionisation process sensitivity reflects the actual MS conditions.

2.3.4 Method choice and validation

2.3.4.1 Method choice

Two of the five methods described in Section 2.3.3 were investigated for the extraction of chlorinated solvents from Scots pine needles based on the availability of GC/ECD, and occasional GC/MS for validation.

Method 1 : Thermal desorption using apparatus designed to reverse the process whereby volatile species enter leaves;

Method 2 : A solvent extraction method adapted for use in the field to reduce laboratory contamination problems.

Results from thermal desorption trials using the method described below and designed apparatus (Appendix 3) showed poor reproducibility with non-quantitative determination of chlorinated solvents. Under the optimised conditions of 1.77 ml/min N_2 flow through the desorption apparatus, recovery varied from 50% to 75% for C_2Cl_4 to $C_2H_3Cl_3$, for example, when spiked (Aldrich standard in methanol) air sampler tubes were analysed.

Due to the success of the field-extraction method developed in parallel, this approach was abandoned at this stage. It is envisaged, however, that a continuation study using a 6-port sampling valve as a series of switching loops to introduce the sample as a 'slug' directly onto the column, via the carrier gas (He) stream, would improve % recovery. The replacement of the stainless steel Dewar loop with a loop of 0.53 mm i.d. deactivated capillary tubing may also reduce loss of volatiles through the trap system.

The solvent extraction method developed for use in the field proved the most successful and was adopted as the technique of choice with capillary GC/ECD.

Solvent extraction method

Sub-samples of about 1g (fresh weight) Scots pine needles of either the 1992 or 1993 year class were scissor-cut (Simonich and Hites, 1994) in the field into pre-cleaned 5ml glass vials (Aldrich). A 2ml aliquot of low-haloform pentane (Rathburn, HPLC) was added using a glass syringe, and the vial sealed with a Teflon-lined silicone rubber septum (Aldrich) and aluminium crimp top (Aldrich). Blanks containing no needle sample were made up in an identical manner. In addition, some needles from both year classes were collected into pre-cleaned glass-stoppered flasks or empty vials and sealed for storage at 4°C (refrigerator) or -20°C (frozen) (Calamari *et al.*, 1994; Morosini *et al.*, 1993; Simonich and Hites, 1994). Upon return from the field, the sealed extraction vials, both samples and blanks, were stored at 4°C for 24 hours and shaken vigorously every 8 hours. Each solvent extraction vial was analysed in triplicate by optimised GC-ECD (Chapter 3, Brown *et al.*, 1995).

All sample preparation was under fumehood conditions. Glassware was rigorously cleaned using chromic acid, washed with detergent, rinsed with de-ionised water and kept in a drying oven at 110°C before use. The scissors used for direct field-cutting of foliage into the vials were meticulously cleaned using purified hexane, and wrapped in aluminium foil before use.

2.3.4.2 Validation of the solvent extraction method

(1) Blank level comparisons

Analysis of field-extracted needle samples taken from Banchory forest (Section 2.2.3), including those stored at 4°C for two months and those frozen for a month before analysis (Calamari *et al.*, 1994), shows that there is up to a tenfold reduction in chlorocarbon blank levels (as reflected in the LOD values) when using field, rather than laboratory, prepared blanks (**Table 2.3.4.2.1**).

In addition, the good agreement between the field-extracted, frozen and 4°C-stored needle samples shown in the Table validates the use of frozen and stored samples for retrospective analyses (Chapter 3).

Table 2.3.4.2.1 Comparison of results for Banchory 1992 year class needles collected in November 1993 and stored under different regimes. All results are corrected for the appropriate blank and are expressed in pg/injection as mean ± standard deviation before conversion to ng/g. The data for $CHCl_3$ are excluded due to laboratory contamination of the GC internal standard (Chapters 3 and 5). Blanks were prepared in the field or laboratory as indicated. The probability plot method (Chapter 5) is used to calculate the limit of detection (LOD) as the field blank mean plus two standard deviations on the mean. The non-detect value (ND) is defined by the slope and intercept of the compound calibration plots (Chapter 5).

		Storage regime									
		field prepared blank							laboratory prepared blank		
		Tree 1	Tree 2	Tree 3	amalg. on site	4°C for 2 months	-20° for 1 month		amalg. on site	4°C for 2 months	-20°C for 1 month
$C_2H_3Cl_3$	LOD	306	306	306	306	306	306	LOD	1104	1104	1104
	ND	25	25	25	25	25	25	ND	25	25	25
	1992	24±70	7±113	-36±66	94±60	-6±28	-13±85		94±60	-517±28	-524±85
	1993	-71±5	-45±29	-66±12	-63±15	no sample	-59±23		-574±15	no sample	-569±23
CCl_4	LOD	99	99	99	99	99	99	LOD	101	101	101
	ND	22	22	22	22	22	22	ND	22	22	22
	1992	2±6	-1±2	-3±6	-3±6	-4±9	-12±15		-16±6	-18±15	-29±24
	1993	5±6	-2±7	-4±15	4±11	no sample	-13±28		-10±11	no sample	-26±28
C_2HCl_3	LOD	75	75	75	75	75	75	LOD	132	132	132
	ND	23	23	23	23	23	23	ND	22	22	22
	1992	39±8	2±1	12±10	17±24	14±14	7±15		-40±24	-43±14	-50±15
	1993	54±33	44±27	21±32	17±7	no sample	18±6		-40±7	no sample	-39±6
C_2Cl_4	LOD	-36	-36	-36	-36	-36	-36	LOD	2	2	2
	ND	-39	-39	-39	-39	-39	-39	ND	-39	-39	-39
	1992	38±4	22±1	23±8	22±19	21±11	16±20		-3±19	-5±11	-10±21
	1993	27±11	33±15	28±16	28±8	no sample	16±16		3±8	no sample	-9±16

(2) The data in **Table 2.3.4.2.2** justifies the adoption of a 24 hour extraction period, as amounts of chlorinated solvents extracted from needles remain constant from 24 to 48 to 72 hours.

Table 2.3.4.2.2 Results of 48 and 72 hour extraction periods expressed as a % difference from the 24 hour result. The substantial increase in $CHCl_3$ observed due to contamination during this time is discussed in Chapter 5.

Chlorinated solvent	Extraction time in hours at 4°C	
	48	72
$CHCl_3$	+12	+38
$C_2H_3Cl_3$	+5	+9
CCl_4	+2	+7
C_2HCl_3	-3	+2
C_2Cl_4	-7	-4

(3) Between-vial reproducibility is demonstrated in **Table 2.3.4.2.3**. Spiking trials are used to further validate the method (Chapter 3).

Table 2.3.4.2.3 Mean ± standard deviation (n=3) results in pg/injection for ten vials each analysed in triplicate. The ten vials are sub-samples of an amalgamated Scots pine 1992 year class needle sample collected from a sampling position at Banchory forest on 09/05/94. Each sub-sample was field extracted using an identical procedure and analysed by optimised GC-ED. The probability plot method (Chapter 5) is used to calculate the limit of detection as the field blank mean plus two standard deviations (LOD) on the mean. The ND value is defined by the calibration plot slope and intercept (Chapter 5).

Vial	Chlorinated solvent			
	$C_2H_3Cl_3$	CCl_4	C_2HCl_3	C_2Cl_4
LOD	25	27	89	-39
ND	25	22	23	-39
1	30±5	23±1	26±3	-24±16
2	28±6	26±1	36±16	-27±21
3	28±4	25±3	27±3	-24±16
4	26±1	27±3	26±3	-31±14
5	30±8	23±2	33±4	-33±7
6	26±2	26±2	25±3	-36±6
7	33±12	24±3	29±5	-31±12
8	26±2	26±2	34±10	-30±15
9	26±2	27±1	25±2	-36±5
10	34±7	22±1	29±8	-23±5
amalgamated sample	29±5	25±1	27±5	-27±18

(4) Representativity studies

Needles from both year classes were collected during May/June 1994 from a further six or nine trees per forest at a height of 10m (Section 2.3.1) to allow a twelve tree representativity study. Individual samples were collected from each of the twelve trees in addition to the normal amalgamated samples to show that chlorocarbon concentrations in the original trees sampled were truly representative of those typical of that sampling site.

Each individual extraction vial was analysed in triplicate, and the optimised GC/ECD results (calculated using the probability plot method described in Chapter 5) are given in **Tables 2.3.4.2.(4-7)**. The results are expressed in pg/injection and are the mean and standard deviation of the triplicate injections before conversion to ng/g (see Chapter 5).

Some of the data used to produce **Table 2.3.4.2.6** is graphically presented in **Figure 2.3.4.2.1**. The example used is concentration of CCl_4 in 1992 needles at Banchory forest.

The Tables and Figure show that no major variation in chlorocarbon concentration is observed between the four trees at each sample point and that the amalgamated sub-sample generally taken is representative of that sample site. The results in **Table 2.3.4.2.7** from a truncated 13m sampling programme at Devilla, using the original three trees, show that as there is no significant difference on this date between the 10m and 13m heights, the 10m sampling can be adopted as representative of both heights for the 12 tree study.

Table 2.3.4.2.4 12 tree representativity study at Devilla forest. 1992 and 1993 year class needles from trees 5m apart collected at 10m height. chlorocarbon concentration in pg/injection, results expressed as mean ± standard deviation, n=3. Trees in **bold** are those from which amalgamated sample is taken. LOD and ND calculated as in Chapter 5.

Position	Tree	Chlorinated solvent				Needle class
		$C_2H_3Cl_3$	CCl_4	C_2HCl_3	C_2Cl_4	
	LOD	27	24	26	-35	
	ND	25	22	23	-39	
1	**1**	25±0.4	23±1	23±0	-39±0	1992
		26±2	22±1	23±0	-39±0	1993
	2	25±0	23±1	24±0.1	-38±2	1992
		25±0	24±0.5	24±1	-39±1	1993
	3	28±3	24±1	23±0.1	-39±0	1992
		25±0.2	24±1	24±0.3	-37±3	1993
	4	26±3	23±1	24±0.3	-39±0	1992
		26±1	24±0.4	23±0	-39±0	1993
2	**5**	26±3	23±0.5	23±0	-37±4	1992
		25±1	24±0.3	23±0	-37±4	1993
	6	25±0	24±0.2	23±0	-35±6	1992
		25±0	24±0.5	24±0.3	-39±0	1993
	7	25±0	25±0.2	23±0	-34±8	1992
		25±0	24±0.1	24±0.2	-33±1	1993
	8	25±0	24±2	24±0.2	-39±0	1992
		25±1	24±1	23±0	-37±3	1993
3	**9**	25±0.2	25±1	23±0	-37±4	1992
		25±0.1	23±1	23±0	-39±0	1993
	10	25±0	22±1	23±0	-39±0	1992
		25±0	22±1	24±0.5	-35±6	1993
	11	26±1	23±1	24±0.2	-34±4	1992
		26±1	23±1	24±0.3	-37±4	1993
	12	26±2	24±1	23±0	-39±0	1992
		25±0	22±0	23±0	-39±0	1993
Amalg.	**1,5,9**	26±3	23±0.3	24±0.1	-37±4	1992
		25±1	24±1	24±0.1	-36±4	1993

Table 2.3.4.2.5 Representativity study at Ponsonby Tarn forest. 1992 and 1993 year class needles collected at 10m height, chlorocarbon concentration in pg/injection. Results expressed as mean ± standard deviation, n=3. Trees in **bold** are those from which the amalgamated sample is taken.

Site	Tree	Chlorinated solvent				Needle Class
		$C_2H_3Cl_3$	CCl_4	C_2HCl_3	C_2Cl_4	
	LOD	25	24	43	-39	
	ND	25	22	23	-39	
1 (West) 0.5 km from BNFL	**1**	25±0	22±1	25±3	-39±0	1992
		25±0	24±2	23±0	-39±0	1993
	2	25±0.5	25±1	24±1	-39±0	1992
		25±0	23±2	25±1	-36±5	1993
	3	25±1	22±1	29±5	-35±7	1992
		25±0	23±1	25±1	-39±0	1993
	4	25±0	23±1	24±0.5	-38±2	1992
		25±0	24±1	23±0	-39±0	1993
2 (Centre) 1.0 km from BNFL	**5**	26±1	24±0.4	25±3	-39±0	1992
		30±9	23±2	23±0	-35±7	1993
	6	28±3	24±1	29±3	-39±0	1992
		31±11	23±0.4	26±5	-36±5	1993
	7	25±0	25±1	23±0	-39±0	1992
		26±2	23±1	26±2	-39±0	1993
	8		no sample	taken		1992
			no sample	taken		1993
3 (East) 1.5 km from BNFL	**9**	25±0	22±1	24±2	-39±0	1992
		25±0	24±0.5	27±7	-39±0	1993
	10	25±0	24±2	23±0	-39±0	1992
		25±0	24±1	23±0	-38±2	1993
	11	25±0	23±1	23±0	-37±4	1992
		25±0	23±1	33±17	-37±4	1993
	12	25±0	23±1	27±7	-37±3	1992
		25±0	23±1	23±0	-39±0	1993
Amalg.	West	25±1	23±2	25±3	-39±0	1992
		25±0	23±1	25±2	-39±0	1993
Amalg.	Centre	25±1	24±1	28±6	-38±1	1992
		27±5	24±1	26±4	-39±0	1993
Amalg.	East	25±0	24±0.4	23±0	-39±0	1992
		25±0	23±1	26±3	-37±4	1993

Table 2.3.4.2.6 12 tree representativity study at Banchory forest, 1992 and 1993 year class needles collected at 10m height, chlorocarbon concentration in pg/injection. Results expressed as mean ± standard deviation, n=3. Trees in **bold** are those from which amalgamated sample at the site is taken. LOD and ND as in Chapter 5.

Position	Tree	Chlorinated solvent				Needle class
		$C_2H_3Cl_3$	CCl_4	C_2HCl_3	C_2Cl_4	
	LOD	25	27	89	-39	
	ND	25	22	23	-39	
1	**1**	28±4	25±3	23±0	-27±21	1992
		25±1	23±0.6	23±0	-36±3	1993
	2	25±0	23±1	25±3	-29±18	1992
		25±0	22±0	23±0	-35±6	1993
	3	25±1	25±0.4	23±0	-39±0	1992
		26±2	24±3	23±0	-39±0	1993
	4	26±2	23±1	27±3	-39±0	1992
		25±0	25±0.3	26±4	-39±0	1993
2	**5**	26±2	24±1	23±0	-39±0.1	1992
		27±3	24±2	30±12	-39±0	1993
	6	25±0	23±2	23±0	-39±1	1992
		25±0	25±1	23±0	-39±0	1993
	7	29±7	23±2	27±7	-39±1	1992
		28±5	26±2	23±0	-35±4	1993
	8	38±5	26±1	34±19	-30±4	1992
		25±0	26±2	23±0	-37±3	1993
3	**9**	27±4	25±0.4	23±0	-35±4	1992
		27±4	26±3	28±7	-34±4	1993
	10	25±0	27±1	23±0	-36±5	1992
		25±1	23±3	23±0	-31±3	1993
	11		sample	lost		1992
		25±0	25±1	23±0	-35±4	1993
	12	27±5	27±0.4	23±0	-36±6	1992
		25±1	26±0.5	23±0	-36±5	1993
	Amal. **1,5,9**	29±5	25±0.6	27±5	-27±18	1992
		27±3	24±2	28±8	-36±5	1993

Table 2.3.4.2.7 Representativity study at Devilla forest, 1992 and 1993 year class needles from original three trees sampled 10m apart collected at 13m height. Chlorocarbon concentration in pg/injection, expressed as mean ± standard deviation, n=3, using the probability plot method to calculate the LOD, and calibration slope and intercept to calculate ND values (Chapter 5).

Tree	Chlorinated solvent				Needle Class
	$C_2H_3Cl_3$	CCl_4	C_2HCl_3	C_2Cl_4	
LOD	27	24	26	-35	
ND	25	22	23	-39	
1		sample	lost		1992
	26±2	29±1	24±0.2	-37±4	1993
2	25±0	25±1	24±0.2	-38±3	1992
	27±3	23±1	23±0	-35±4	1993
3	25±0	22±1	24±0.2	-38±3	1992
	28±5	23±1	23±0	-37±4	1993
amalg 1,2,3	25±0	27±1	24±0.3	-37±3	1992
	29±4	26±3	24±0.2	-35±4	1993

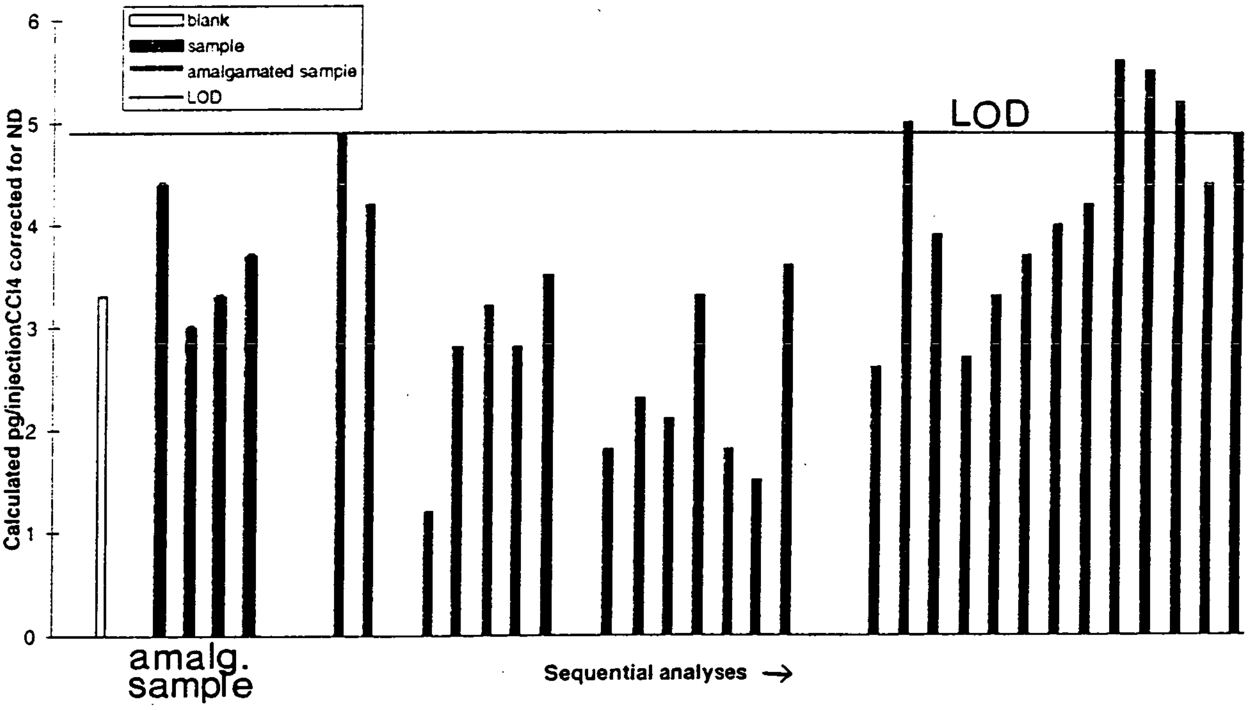


Figure 2.3.4.2.1 Banchory 11 tree representativity study for CCl_4 content of 1992 needles collected on 09/05/94. All the sample and blank pg/injection values, before the mean calculation, are shown. These values are corrected for the non-detect (ND) value, and the LOD (Limit of Detection : field blank mean plus two standard deviations on the mean; Chapter 5) is shown. The amalgamated sample results are representative of the sample site.

Chapter 3

Experimental : Gas chromatographic analysis

The difference between Art and Science is that Science is what we understand well enough to explain to a computer. Art is everything else.

Donald Knuth

3. EXPERIMENTAL: GAS CHROMATOGRAPHIC ANALYSIS

3.1 Introduction

Chromatographic methods have been widely used in the study of volatile organic compounds in a variety of environmental matrices. (Fox, 1991; Frank and Frank, 1989; Sack *et al.*, 1992). Such studies include the determination of chlorinated solvents in the atmosphere and plant foliage (Rudolph *et al.*, 1986).

'Chromatography' is a general term for separation processes in which the components of a mixture are repeatedly equilibrated between two phases; normally one of the phases is fixed (stationary) and the other is mobile (Jennings, 1980). Gas-liquid chromatography (GC) uses a mobile gaseous phase and a liquid stationary phase. The key to effective analysis of substrates in any matrix is the careful and meticulous optimisation of all the GC system parameters for a particular analysis (Jennings, 1987).

Gas chromatography/mass spectroscopy (GC/MS) is a widely used tool for confirming the identity of separated constituents, the primary limiting factor for routine operation being one of expense (Mitchum, 1991).

The equipment earmarked for this project consisted of :

(i) A dual channel GC-Autosystem (Perkin Elmer) without autosampler, fitted with a ^{63}Ni electron capture detector (ECD) and a flame ionisation detector (FID). Injection is onto packed or capillary columns via a split / splitless injector;

(ii) A Perkin Elmer integrator (type 2010) with software for method setup and control, data handling and storage. Peak areas acquired are converted into amounts using response factors generated from previously determined calibration analyses (Chapter 5).

Following instrument installation, a GC/ECD method was developed, optimised, calibrated and validated for chlorinated solvent determinations based on previous studies and theoretical and practical considerations (**Table 3.1.1**).

Table 3.1.1 Examples of literature methods for chlorinated solvent determination in different matrices, with the analytical conditions and detection limits achieved.

Matrix	Method	Injection	Stationary phase	Conditions	Detection limits achieved	References
Spruce needles	Extraction with hexane followed by GC/Chemical Ionisation Mass Spectrometry	On column 1μl syringe	1m x 0.25m fused silica retention gap 25m x 0.35mm 2.0μm dimethylpolysiloxane column (PS255/DB5)	He (8.7psi, 60kPa inlet) 55°C for 1min, 20°C/min to 150°C. Data acquired after 2.5min. 1m x 0.1mm fused silica silanized interface to MS. 100eV electron energy, emission current 0.2mA, multiplier 2000V, ion source 250°C.	C_2Cl_4 100pg/g needle	Frank and Frank, 1989
Spruce needles	Mulching in ethyleneglycolmonomethylether, ultrasound and vacuum condensation GC/ECD	Split 2μl syringe	50m x 0.53mm CP sil 5CB chemically bonded	Ar/Methane 95/5@ 10 ml/min. Split 45 ml/min 60°C isothermal 15 min, 5°C/min to 135°C. 0.1min isothermal, 40°C/min to 250°C. ECD 320°C. Makeup Ar/methane 95/5 @ 50 ml/min.	$CHCl_3$ 3ng/g $C_2H_3Cl_3$ 1ng/g CCl_4 2ng/g C_2HCl_3 2ng/g C_2Cl_4 3ng/g	Diezel *et al.*, 1988

Matrix	Method	Injection	Stationary phase	Conditions	Detection limits achieved	References
Air	On-site sampling/ thermal desorption onto Haysep®/ carbosphere®. 10 port valve for switching. GC/ECD.	Automated. 40ml drawn onto adsorbent.	28m x 0.3mm 2.0μl crosslinked SE54	ECD 275°C. 0.1μsec, reference current 2nA. Ar/Methane (9+1) makeup @ 30 ml/min.	$CHCl_3$ 0.02 μg/m^3 $C_2H_3Cl_3$ N/A CCl_4 N/A C_2HCl_3 0.02 μg/m^3 C_2Cl_4 N/A	Frank *et al.*, 1991a Frank *et al.*, 1991b
Air	Tenax TA®/charcoal adsorbent trap. Thermo-desorption/ cryofocussing to GC/ECD	10ml/min flow into cartridge. Flash vaporisation.	8μm PS255 polydimethyl-siloxane crosslinked with dicumyl peroxide	H_2 carrier. Inlet pressure 25kPa. oven at 25°C 2mins isothermal; 10°C/min to 100°C. Liquid N_2-cooled cryotrap (-130°C) for 25mins; heat to 200°C in 30 seconds. ECD constant current Ar/Methane make-up (9+1) @ 30ml/min.	blank values $CHCl_3$ 9.0±8.0 ng/m^3 $C_2H_3Cl_3$ 2.5±0.9 ng/m^3 CCl_4 0.8±0.2 ng/m^3 C_2HCl_3 nd C_2Cl_4 1.8±0.5ng/m^3	Frank and Frank, 1988b
Air	Tenax TA® thermo-desorbed to GC/ECD.	700ml total volume sampled @ 35ml/min	30m x 0.32mm 1.8μm DB624 Injection port 250°C	He carrier 1ml/min. oven @ -20°C to trap volatiles (liquid CO_2). Flash vaporised to 230°C with He flush. ECD @ 300°C, N_2 makeup gas @ 30ml/min.	$CHCl_3$ 0.6 ng/m^3 $C_2H_3Cl_3$ 0.6 ng/m^3 CCl_4 1.3 ng/m^3 C_2HCl_3 2.4 ng/m^3 C_2Cl_4 0.4 ng/m^3	Sturges and Taylor, 1990

3.2 GC method development

3.2.1 Choice of GC system components

Detector

The ECD (electron capture detector) is chosen on the basis of its greater sensitivity, lower detection limit and selectivity for electronegative elements within molecules. The linear range of the ECD is, however, smaller than that of the FID (10^3 compared to 10^6-10^7).

The radioactive foil source of the ECD (^{63}Ni) emits β particles and ionises the carrier gas flowing through the detector forming a plasma containing some thermal electrons. A constant current is set up under the influence of a polarising electric field, as the thermal electrons are captured. In the form of ECD used in this study, the background current resulting from capture of the thermal electrons is used as a baseline value. Electrophilic species entering the cell capture thermal electrons. The amount of loss of detector background current is translated as a detector signal which is integrated and inverted to form a corresponding peak.

The amount of current reduction is a function of compound concentration and electrophilic nature. The ECD is a concentration - sensitive detector, i.e. sensitivity is inversely proportional to make-up gas flow. The make-up gas (N_2) is used to increase flow through the detector to 30 - 50ml/min, carrier gas flows are usually in the range of 1-20ml/min. The ECD is non-destructive to the sample allowing subsequent mass spectroscopy.

Injector

The sample must be introduced as the narrowest possible band at the start of the column. Split/splitless injections are flash-vaporisation modes which may be preceded by cold trapping. In this study, splitless injection was used with all the sample entering the column at very low flow rates (1-3 ml/min). Samples were in 1μl aliquots and injection was done manually through a self-sealing silicone rubber septum.

Syringe techniques offer 3% accuracy at best (Rood, 1991). Syringe discrimination of high boiling point components can occur, these remain behind on the needle walls after volatile components have vaporised. Operator technique is critical; the syringe was depressed at a constant rate 0.1min after insertion. Loss of sample volatiles can be observed if there is a delay between syringe filling and injection, and ghost peaks can arise if sample is trapped in the rubber septum.

The *solvent effect* was exploited to re-concentrate elution bands at the front of the column by increasing values of *k* (partition ratio : distribution of molecules between the stationary and gaseous phases - Appendix 4). The sample solvent condenses in a film (the wet zone) and vaporised sample components are 'trapped' in this extension to the stationary phase. A large decrease in column diameter and phase ratio is observed, with concomitant increase in *k* - the number of molecules in the gaseous phase is greatly reduced (Appendix 4). The solvent effect depends on the initial column temperature. This was set at 35°C, which was the practical lower limit at ambient temperature. The condensation of hexane (bp = 69°C) was therefore more marked than that of pentane (bp 36°C), and consequently this extraction/injection solvent was more effective at trapping and re-focusing solute bands. The boiling point of hexane, is, however, higher than that of $CHCl_3$ (60.5-61.5°C) and only marginally less than that of CCl_4 (77°C), which limits the focusing advantage of this solvent for these components. Pentane is considerable more effective as a wet zone solvent in these cases (Rood, 1991).

Carrier gas

Helium was adopted on the basis of Van Deemter plots which displayed reasonably flat curves indicating a broad range of minimum values and wide velocity window (Appendix 4). Higher flow rates than for N_2 can be adopted without sacrificing efficiency and good separation over a wide range of eluting temperatures. Analysis times were reduced compared to N_2 and the lower cost and risk factors when compared to H_2 are advantageous. Oxygen and moisture scrubbers need to be installed as the minimum requirement for He purity is 99.995%.

Column

The choice of stationary phase is the most important factor during column selection. A cross-linked and bonded SE-54 (5% phenyl substituted, Alltech UK) column, of 30m length, 0.32mm ID and 1.0μm film, was chosen based on the following discussion and citations in Table 3.1.1.

Phase type

Stationary phase polarity is dependent on the type and amount of functional group substitution of the (generally) polysiloxane backbone. The least polar phase that can do the job should be used to prolong column lifetime, and matching stationary phase with the solute polarity is advised. The SE-54 (DB-5 equivalent) has 5% phenyl subsitution which is helpful when separating components of similar boiling points (**Table 3.2.1.1**).

Different types of interactions can occur between solute and stationary phase. The most important type, hydrogen bonding, is not seen significantly in halocarbons - usually O or N is required to create an electron deficient H atom. Dipole interactions are important for chlorocarbons, contributing to the time spent in the stationary phase ($t_{R'}$) and consequently k. An electrostatic field is set up by centres of +ve and -ve charges resulting from displacement of bonding electrons by induction or resonance, which attract charges formed in a similar way in other molecules. Dipole induction can occur when molecules have polarizable electrons which are brought into the electrostatic field of polar molecules (e.g. π-electrons of SE-54 phenyl ring). Dispersion forces arise from nuclear and electronic vibrations which lead to oscillating dipoles. These are cancelled over the molecule as a whole, but induce temporary oscillating dipoles in neighbouring molecules which vibrate in phase. This is a relatively weak effect with elution occurring in order of increasing solute boiling point. Acid - base reactions are of low importance.

Table 3.2.1.1 Selected stationary phase interaction indices

Stationary phase		Dispersion index	Dipole index	Bascicity index	Acidity index
increasing	DB1	9	0	0	0
polarity &	DB5 / SE-54	11.6	0	0	1
substitution ↓	DB624	10.4	11	3	0

Jones Chromatography, 1990; DB624 = cyanopropylphenyldimethylpolysiloxane.

With increasing substitution, there is a corresponding upper temperature limit that a phase will tolerate;

DB-1	-60°C - 325$^{(1)}$/350°C$^{(2)}$
SE-54	-60°C - 325/ 350°C
DB-624	-20°C - 260°C$^{(1,2)}$

$^{(1)}$isothermal limit, $^{(2)}$temperature programmed limit

The column was conditioned after installation by programming at 10°C/min to 300°C. The cross-linked and bonded (to reduce bleed) SE-54 phase was adopted as being forgiving in novice hands, relatively inexpensive, having a long lifetime, and being less susceptible to degradation.

Film thickness, d_f

A d_f value of 1μm is an acceptably thick film with good efficiency, low β (phase ratio : volume occupied by gas/volume occupied by stationary phase - Appendix 4) and reasonable capacity (250-600ng per injection).

Column diameter, $d = 2r_o$

The 0.32 mm ID was adopted on the basis of its separating power (Appendix 4), good sample capacity, low column bleed and recommended use for splitless injection. The value of β was 80 (Section 3.3), columns with $\beta \leq 100$ are suited for analysis of volatiles (Rood, 1991).

Column length, L

A 30m column length was used based on Van Deemter evaluation of the partition ratios of the compounds of interest (k=0.6 CH_3I, k = 7 C_2Cl_4). A longer column is not practical in terms of pressure drop. In addition, the increase in N (number of theoretical plates) with length may be useful, but the variation in resolution with $N^{1/2}$ and greater column bleed and expense are disadvantageous (Appendix 4).

3.2.2 Operating conditions

The total gas flow through the ECD was set to 45 ml/min, the carrier gas (He) was of minimum purity 99.995% with initial flow rate of 1.03 ml/min at the optimum pressure of 6.4 psi. The N_2 make-up gas purity was 99.95%. ECD and split gas flows were measured using a soap bubble flow meter attached to the detector or split vent and the built-in AutoSystem stopwatch. A calibrated carrier gas pressure read out (kPa and psi) was also available. The ECD temperature was set at 375°C to reduce detector contamination caused by the condensation of high boiling point sample residues. Conditioning the ECD at 450°C with increased N_2 make-up to a total gas flow through the detector of 60-100 ml/min was used if background levels were >8mV.

The injector temperature was set at 150°C to reduce loss of volatiles in the injection port. Splitless injector liners were used unpacked to reduce sample mixing which results in dilution of the vaporised sample with carrier gas, and broader peaks. Over 200 injections at moderate temperature could be performed before injection port septa (Teflon-faced microsep, Perkin Elmer) needed to be replaced.

The AutoSystem integrator was MS DOS compatible with a 1MB RAM, a 20MB hard disk for data storage and a 720kB floppy disk drive for data archiving or transfer. The integrator input channel voltage range was matched to the output voltage of the ECD. An attenuation of 16 was used to ensure GC output was above the configured integrator noise level, i.e. the maximum detector signal producing a 1 volt output to the detector = 38.0 kHz. Attenuation of the detector signal before transfer to the integrator was carried out under actual operating conditions prior to running the first sample.

After capillary column installation, a blank run using the temperature programme adopted was stored as a baseline profile. This baseline profile was automatically subtracted from the signal generated during each subsequent GC run to provide background compensation for column bleed.

A 1m, deactivated fused silica (0.25mm ID, Alltech, UK) retention gap was attached to the injector end of the column using a gas tight glass press-fit union (Chrompak, UK). This inexpensive pre-column had two main functions :

(1) As a site for solvent condensation during the solvent effect, eliminating localised stripping of the SE-54 phase due to high solvent concentrations;

(2) To protect the column from the deposition of non-volatile sample residues.

3.2.3 Integrator method for data acquisition

The data collection parameters include calculated optimum base and area sensitivity parameters (693 and 34), which specify how large a signal must be in order to be detected as a peak rather than noise and when the chromatograph signal is on the baseline. The peak width range setting (1.4-3.2 sec) covered the width at base of the narrowest peak in the run (2.6 sec). This value (fixed) specifies the data collection rate at 10 points per second (14 - 32 data points per peak). Peak integration used a basepoint - to -basepoint baseline construction.

Each chromatogram collected was stored in a unique file. A hard copy was automatically printed on an OKIDATA 320 dot matrix printer (Appendix 5) of ECD mV reading (*y* axis) Vs retention time (*x* axis) plot. Some sample peaks required data reprocessing, for example by manually defining integration start and end of a particular peak and re-integrating, to eliminate artefacts. Artefacts can arise from such sources as baseline abnormalities obscuring the peak of interest or carry-over peaks. Such data were excluded from further analysis (Chapter 5).

3.3 Method optimisation

3.3.1 Solvent purification

To reduce component detection limits to as low a level as possible, the analytical matrix blank levels must also be as low as possible (Frank and Frank, 1988a; Chapter 5). Only solvents of very high purity must be used for extraction, injection, and calibration/internal standard preparation. Solvent contamination levels, either intrinsic or arising from exchange with laboratory air, form the primary origin of the high field blank detection limits (Black, 1991).

The solvent purification strategy initially focused on hexane as the extraction solvent of choice (Frank and Frank, 1989). The technique adopted by Frank of refluxing for 10 days over sodium suspension prior to distillation was modified. Sodium wire was replenished after 5 days of refluxing the hexane, different solvent suppliers were investigated and an alumina column filtration system used before the reflux (middle 70% collected) (Perrin and Armarego, 1988). **Table 3.3.1.1** shows the method

Table 3.3.1.1 Solvent blank levels in pg/μl after purification expressed as mean ± standard deviation (n=6). nd = lower than detection limit of ECD.

	Purification procedure							
Chlorinated solvent	HPLC grade hexane, as supplied (Aldrich)	HPLC grade hexane (Aldrich) refluxed over Na wire for 10 days, 70% of distillation fraction collected, Na wire replaced after 5 days.	As before, + H_2O wash and dried with Na_2SO_4	HPLC grade hexane, as supplied (Aldrich) after passing through alumina column	HPLC grade hexane (Aldrich), alumina column and reflux over 2 x Na	Oekanal hexane as supplied (Reidel de Haen)	Oekanal MeOH as supplied (Reidel de Haen)†	Rathburn pentane as supplied (low haloform)‡
$CHCl_3$	15.1±1.0	1.18±0.28	1.03±0.17	1.71±0.30	5.18±0.52	0.80±0.61	1.65±1.09	nd
$C_2H_3Cl_3$	38±28	8.92±0.67	10.17±0.40	510±107	0.82±0.21	1.9±1.5	3.05±0.09	0.41±0.32
CCl_4	5.1±4.0	0.78±0.05	0.79±0.01	8.68±0.68	0.83±0.47	0.10±0.01	nd	nd
C_2HCl_3	15.±7.6	15.3±3.2	13.65±0.50	17.7±4.3	12.4±6.2	14.7±10.2	14.6±4.7	7.3±2.5
C_2Cl_4	19.1±7.7	18.7±10.0	19±12	27.4±3.3	nd	16.5±0.46	9.0±7.9	0.67±0.09

† adopted as solvent for preparing calibration standards

‡ adopted as extraction solvent for foliage samples and passive air samplers

progression towards reduced detection limits. Pentane (low haloform, Rathburn) was eventually adopted as the extraction solvent based on low blank levels and superior chlorocarbon extracting properties. Oekanal methanol (Reidel de Haen) was used to dilute the Aldrich standard obtained for calibration purposes (Section 3.3.3). As expected, typical field blank levels were found to be considerably higher than those of the solvent, particularly for $CHCl_3$ (Chapter 5).

3.3.2 Internal standard preparation

Changes in peak area per standard injection can be attributed to one of two sources:

(a) Errors arising from the total amount injected per injection, or;

(b) Daily variation in ECD response.

Expressing the area response relative to an internal standard reduces the uncertainty in either case. In situation (a), there is less quantitative variability on an injection-to-injection and day-to-day basis than direct area counts, while in situation (b) the ECD response will be proportionally reduced or increased for both internal standard and peak of interest. In practice (b) was found to be the largest source of uncertainty (Chapter 5). Iodomethane (CH_3I) was chosen as the internal standard because of its short retention time, elution away from any of the peaks of interest (unlike other compounds investigated such as $C_2H_2Cl_4$) and its similar physical properties as a volatile C_1 halocarbon.

A solution of 1.935 μg/ml internal standard in methanol (Reidel de Haen) was made up in a clean Teflon-lined septum-sealed sample vial by diluting 5μl of 3.87×10^{-4} g/ml solution (CH_3I supplied by Aldrich) into 1ml Reidel de Haen methanol. Each 1μl injection of sample was spiked with 0.1μl of this solution, thus the amount of CH_3I per injection was constant at 194 pg (± error in measuring volume injected). All chlorinated solvent data were expressed in terms of the ratio of peak area of solvent to peak area of the internal standard (the Internal Standard Ratio, or ISR).

Concentrations of CH_3I in the atmosphere are very small, the reported global average is 0.8 pptv (Rasmussen *et al.*, 1982; Chapter 1), even though it is thought to be the dominant gaseous organic iodine species in the troposphere (Tsetsi *et al.*, 1989). The internal standard concentration used was several orders of magnitude greater than this, and it would therefore be expected that if concentrations were present in the pine needles or air sampler leaf wax tested, these would fall within the limits of variability of the analytical method and would not be detected as significant (Chapter 5).

3.3.3 Calibration standard preparation

A gravimetrically prepared primary standard including the five chlorinated solvents of interest at 100μg/ml in pesticide grade methanol (calibration standard 38,509-3, purgeable halocarbon mix for EPA method 601, Sigma Aldrich, UK) was used (Frank *et al.*, 1991a). The primary standard was successively diluted using ultrapure methanol (Oekanal, Reidel de Haen) to yield secondary standards over the range 2.5 pg- 1.0 ng per 1μl volume. Each standard was made up containing 194 pg/injection of CH_3I as internal standard.

Six calibration concentration levels were used to generate the plots used in statistical analysis of the peak area data (Chapter 5). These were at 500, 360, 270, 180, 90 and 40 pg/μl of each component. Six level calibrations were run approximately every two months, and three stage (180, 90 and 40 pg/µl) approximately every month. The 180 pg/µl standard was used as a day-to-day check to test for systematic errors (refer to Chapter 5).

3.3.4 Detection limits

The limits of detection determined are shown in **Table 3.3.4.1**. The probability plot method used to calculate the limit of detection for determination of sample concentration is discussed in Chapter 5.

Table 3.3.4.1 ECD limits of detection (pg per injection) with lowest literature values for comparison. RG = retention gap.

Chlorinated solvent	**DB1 (10m)+ RG(1m)**	**SE54 (30m)+ RG(1m)**	**literature** (refer to Table 3.1.1)
$CHCl_3$	47.0	14.6	1.0
$C_2H_3Cl_3$	5.2	1.4	0.4
CCl_4	6.4	0.3	0.2
C_2HCl_3	37.2	4.6	1.0
C_2Cl_4	4.1	0.8	0.3

3.3.5 Temperature programming

The oven operating conditions were established as a compromise between maximum resolution (R_s) and minimum run time at the OPGV (**Table 3.3.5.1**, refer to Appendix 4). The resolution (as a measure of component separation) is determined as twice the difference in retention time between the two components of interest divided by the sum of the peak width at base of the two peaks (Appendix 4). The critical separation between CH_3CCl_3 and CCl_4 is taken as representative of column performance.

Table 3.3.5.1 Optimisation of oven temperature ramping and R_s. RG=retention gap.

Column system	Oven temperature programme	R_s
DB5 (30m) + RG (1m)	30°C 10 min hold, 5°C/min to 70°C, 10 min hold. 10°C/min to 260°C, 10min hold	1.48
SE-54 (30m) + RG (1m)	30°C 10 min hold, 5°C/min to 70°C, 10 min hold. 10°C/min to 260°C, 10 min hold	4.01†
SE-54 (30m) + RG (1m)	35°C 8 min hold, 6°C/min to 120°C, 10 min hold. 30°C/min to 290°C, 10 min hold	2.43‡

† system optimised for maximum R_S, ‡ system optimised for minimum runtime.

3.3.6 Van Deemter treatment

The value of u_{opt} and the OPGV are determined using N_2O as an unretained solute with a sharp symmetrical peak (0.5cm^3 injection). For example, on a 30m SE-54 (+1m RG) column at a carrier inlet pressure of 6.4psi, N_2O elutes after 143.94 seconds (t_{N2O}), $u=L/t_R$ = 3100/143.94 = 21.53 cm/sec. At a lower head pressure, t_{N2O} is longer, e.g. at 5.9 psi He, u = 3100/165.4 = 18.74 cm/sec. The velocity window plots (**Figure 3.3.6.1**) based on the most (C_2Cl_4, high k) and least retained solutes (CH_3I, low k) plotted using the data from **Table 3.3.6.1** show the variation in u_{opt} for the different compounds and that the OPGV range (1.25-2 x u_{opt}) = 22.55-36.08 cm/sec corresponds to 6.5-8.5 psi He carrier head pressure. The literature recommends a linear flow rate of 20-35 cm/sec (8psi) for a 0.32mm ID, 30m column (Rood, 1991).

The experimental values determined for parameters including *n*, *k* and *β* under optimised conditions are listed in **Table 3.3.6.2**. A linear flow rate of 21.53 cm/sec corresponding to 6.4 psi He was actually used (1.2 x u_{opt}) to benefit both from the improvement in efficiency with temperature increase and to improve the difficult CH_3CCl_3 to CCl_4 separation.

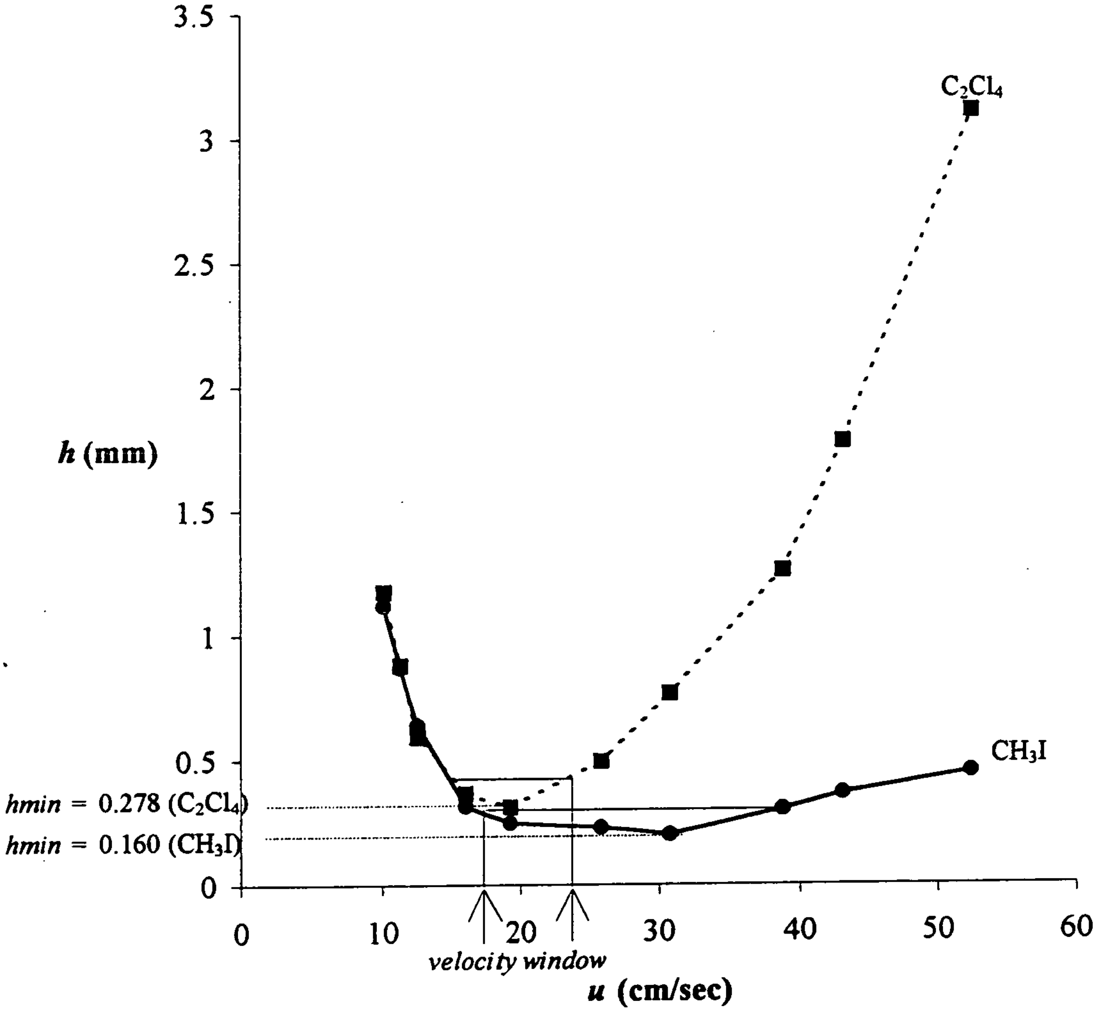


Figure 3.3.6.1 Velocity window plot at 40°C isothermal optimised conditions, SE-54 + RG with 10% permitted increase in h_{min} (Appendix 4).

Table 3.3.6.1 Linear velocity at different head pressures with corresponding *h* and *n* for CH_3I and C_2Cl_4 as the least and most retained solutes of interest (refer to Appendix 4 for equations).
Data from this Table were used for the velocity window plot.

He psi	t_{N2O} (mins)	u (cm/sec)	n_{CH3I}	h_{CH3I}	n_{C2Cl4}	h_{C2Cl4}
1.0	5.110	10.103	27778	1.116	26428	1.173
2.0	4.564	11.32	35673	0.869	35348	0.877
3.0	4.120	12.54	48589	0.638	52365	0.592
5.0	3.227	16.01	99359	0.312	85165	0.364
6.0	2.693	19.19	126531	0.245	99679	0.311
8.0	2.000	25.83	135965	0.228	62880	0.493
9.5	1.680	30.76	155000	0.200	40576	0.764
11.0	1.333	38.76	103679	0.299	24662	1.257
12.5	1.200	43.06	84932	0.365	17435	1.778
16.0	0.987	52.35	69042	0.449	9971	3.109

Table 3.3.6.2 n, k and β values for two different column systems

Parameter	DB-5 (10m, 0.53mm ID, 2.65μm film) + RG (1m)	SE-54 (30m, 0.32mm ID, 1.0μm film + RG (1m)
β	50	80
n (k=7) at u_{opt}/h_{min}	23,900	111,500
h_{min} (k=7)	0.461	0.278
t_{N2O} u_{opt}	0.894 min (2.4 psi)	2.864 min(5.6 psi)
OPGV	0.518 min (3.8 psi)	2.399 min (6.4 psi)
u_{opt}	20.5 cm/sec (2.4 psi)	18.04 cm/sec (5.6psi)
OPGV	25.63-41.00 cm/sec range (av. 33.32 cm/sec @ 3.6psi) 35.4 cm/sec (3.8 psi) used	22.55-36.08 cm/sec range (av. 29.32 cm/sec @ 7.8psi) 21.53 cm/sec (6.4 psi) used
CH_3I	0.6 (t_R = 0.810 min)	0.5 (t_R = 3.535 min)
$CHCl_3$	2.0 (t_R = 1.577 min)	1.6 (t_R = 6.005 min)
$C_2H_3Cl_3$	2.8 (t_R = 1.973 min)	2.1 (t_R = 7.336 min)
k CCl_4	3.3 (t_R = 2.227 min)	2.5 (t_R = 8.264 min)
(at OPGV) C_2HCl_3	4.5 (t_R = 2.863 min)	3.6 (t_R = 10.750 min)
C_2Cl_4	10.5 (t_R = 5.953 min)	6.9 (t_R = 18.383 min)

The lower limits of detection, higher value of n (improved efficiency) and improved resolution (**Table 3.3.5.1**) of the SE-54 column confirm it to be the logical choice.

3.3.7 ECD Linearity

Detector response curves over the range of calibration standards are rectilinear (**Figure 3.3.7.1**) for this ECD operated under optimised conditions. The upper limit of linearity varies between compounds and different column systems (**Table 3.3.7.1**) and above these values, the curves flatten out and quantitative determination is highly inaccurate. Linearity limits determined are similar to those reported in the literature (Frank and Frank, 1988a).

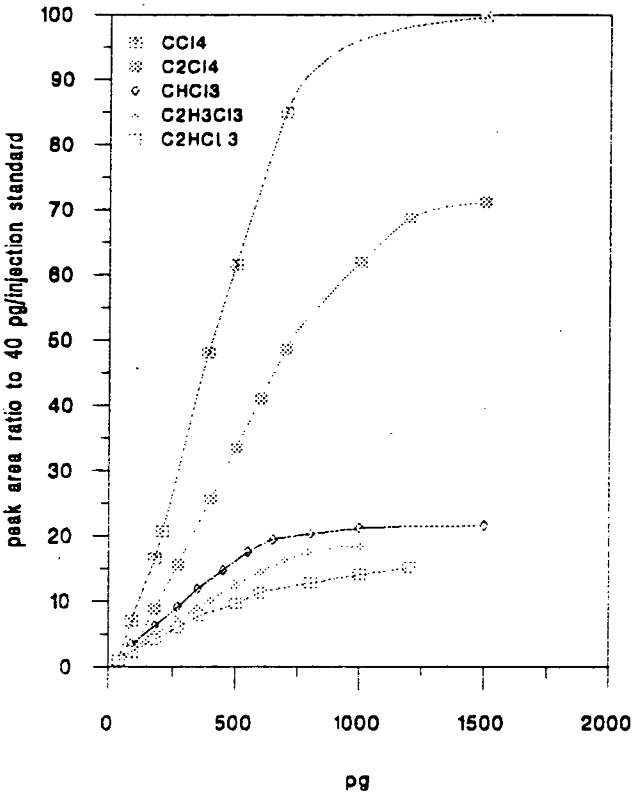


Figure 3.3.7.1 Linearity of ECD response curves on an SE-54 + RG column at optimised operating conditions.

Table 3.3.7.1 ECD limits of linearity (pg per injection) for two column systems.

Chlorinated solvent	DB5 (10m) + RG (1m), 0.53mm ID, d_f=2.65µm, 3.8psi He	SE-54 (30m)+ RG (1m), 0.32mm ID, d_f=0.1µm, 6.4psi He
$CHCl_3$	1,000	700
$C_2H_3Cl_3$	700	800
CCl_4	300	600
C_2HCl_3	1,000	600
C_2Cl_4	500	700

3.3.8 Validation of method

The Aldrich standard reference chromatogram (purge and trap analysis) confirmed chlorocarbon elution order to be $CHCl_3$, $C_2H_3Cl_3$, CCl_4, C_2HCl_3, C_2Cl_4. Spiking experiments were carried out to determine recovery and confirm retention time assignments. Standard extraction vials containing 1g Scots pine needles were spiked with 0.5ml of secondary standard. The extraction volume was made up to 2ml using pentane as before. The vials were stored at 4°C for 24 hours before GC/ECD analysis. Average recoveries from the needle matrix are expressed relative to identical unspiked vials (n=3) and show that a variation of 7 - 31% exists about the expected value (**Table 3.3.8.1**), which is within the acceptable limits of 70-130% recovery (Mitchum, 1991).

The data in **Table 3.3.8.2** confirm that retention time assignments based on elution order and spiking experiments were correct, and were stable to an acceptable ±1% over analyses performed during a 12 month period (Rood, 1991). Note the change in retention times after changing the analytical column (indicated by a break in the Table). Peak area stability over this time (as ISR) is discussed in Chapter 5.

Table 3.3.8.1 Recoveries and retention times of Scots pine needle samples spiked with a secondary standard containing 194 pg/µl CH_3I, 1µl injection to GC/ECD.

Chlorinated solvent	Conc^n of secondary standard (pg/µl)	pg/injection of chlorocarbon from unspiked sample (mean ± sd)	pg/injection of chlorocarbon expected (spiked + unspiked)	pg/injection of chlorocarbon recovered from matrix	% recovery
$CHCl_3$	170.6	51±24	222±24	248.9±17.3	112±14
$C_2H_3Cl_3$	15.6	4.7±0.9	20.3±0.9	26.6±1.4	131±9
CCl_4	12.3	3.8±0.5	16.1±0.5	18.7±0.1	116±4
C_2HCl_3	180.7	54±12	235±12	259±10	110±7
C_2Cl_4	9.3	17.9±3.2	27.2±3.2	29.06±0.58	107±13

Table 3.3.8.2 Retention time stability of the 180pg/μl standard over an 8 month and 4 month period on an SE-54 column under optimised analytical conditions.

Date of analysis	Retention time (mins)					
	CH_3I (internal standard)	$CHCl_3$	$C_2H_3Cl_3$	CCl_4	C_2HCl_3	C_2Cl_4
19/11/93	3.535	6.012	7.350	8.360	10.777	18.693
18/12/93	3.547	5.985	7.332	8.248	10.772	18.698
18/01/94	3.546	5.970	7.363	8.210	10.625	18.587
19/02/94	3.550	5.933	7.337	8.267	10.738	18.650
19/03/94	3.522	6.061	7.358	8.282	10.793	18.705
18/04/94	3.538	5.970	7.297	8.210	10.690	18.628
19/05/94	3.537	6.017	7.323	8.236	10.793	18.686
14/06/94	3.543	6.012	7.412	8.270	10.865	18.738
20/07/94	3.057	5.780	7.073	7.958	10.367	18.403
19/08/94	3.151	5.765	7.105	7.945	10.407	18.387
19/09/94	3.173	5.812	7.020	8.073	10.387	18.343
19/10/94	3.055	5.783	7.062	7.938	10.333	18.362
mean over 50 analytical days during period 19/11/93 to 14/06/94	3.535 ±0.011 (±0.3%)	6.005 ±0.0385 (±0.6%)	7.336 ±0.035 (±0.5%)	8.264 ±0.048 (±0.6%)	10.750 ±0.064 (±0.6%)	18.681 ±0.040 (±0.2%)
mean over 50 analytical days during period 20/07/94 to 19/10/94	3.061 ±0.044 (±1.4%)	5.762 ±0.064 (±1.1%)	7.050 ±0.034 (±0.5%)	7.981 ±0.066 (±0.8%)	10.335 ±0.048 (±0.5%)	18.383 ±0.033 (±0.2%)

= = installation of new SE-54 column

The operating conditions used in the fully optimised method for the bulk of the analyses are summarised in **Table 3.3.8.3**.

Table 3.3.8.3 Optimised GC/ECD operating conditions used for calibration and determination of chlorinated solvent concentrations in needle and air sampler wax samples.

System component	Operating conditions
ECD	375°C ; 45 ml/min N_2
Injector	Splitless, 150°C ; 1µl injection volume
He carrier gas	6.4psi (flow rate 1.03 ml/min) u = 21.53 cm/sec (±1%)
Column	SE-54 (0.32mm ID, 1.0µm film, 30m) + retention gap (1m, 0.25mm ID, deactivated silica)
Oven	35°C 8 min, 6°C/min to 120°C, 30°C/min to 290°C, 10min.

3.4 Gas chromatography/mass spectrometry

3.4.1 Introduction

The use of capillary GC/MS as a highly selective confirmatory tool for quantitative and qualitative environmental analysis is well established (Mitchum, 1991). Complementary mass spectrometry to the GC/ECD analysis results was carried out by Mr A. Newton over 4 days, assisted by myself, at the Scottish Agricultural Scientific Agency (SASA), East Craigs, Edinburgh. The instrument used was a Finnegan MAT (TSQ700) triple stage quadrupole with ICL procedure for instrument control and data acquisition, manipulation and interpretation. Capillary GC separation was on-line using an SE-54 column (30m, 0.32mmID, 1μm film). Ionisation options available included electron impact ionisation (EI), negative chemical ionisation (NCI) and positive chemical ionisation (PCI) with single stage and MS/MS mass separation. Further use was limited by expense and instrument / operator availability.

3.4.2 Background

Mass spectrometry is based on the production of +ve or -ve charged species (ions) from neutral molecules. The mass and relative abundance of these ions formed are characteristic of the compound, i.e. an unambiguous identification 'fingerprint'.

Several types of mass spectrometer exist. All include a device to introduce the sample, an ionisation source, a mass analyser in which ions are separated when moving through an electric or magnetic field on the basis of their mass:charge ratio (m/z), and devices for ion collection, amplification and detection. Compound separation by GC is essential in complex environmental matrices where co-extractives often interfere, the resolving power (ability to separate ions of differing m/z) would have to be proportionally greater if there were sole dependence on separation by mass.

3.4.2.1 MS Operation

The quadrupole mass spectrometer uses a mass analyser consisting of a set of four rods with cylindrical cross section arranged as the parallel sides of a rectangle. A radiofrequency (RF) and DC potential are imposed upon the quadropole rods to form a 3-D, time-varying field. The ions are accelerated from the ion source into this field along the longitudinal axis perpendicular to the rod's surface. The oscillating ion path through this field is dependent on the initial momentum, the RF and the magnitude of the DC component of the RF (Dawson, 1976). Controlling the ratio of RF to DC voltage allows ions exiting the quadrupole filter with a certain m/z to be selected, assuming consistent ionic charge and velocity. These ions traverse the length of the quadrupole region without colliding with the rods. The quadrupole is scanned by ramping the DC component and superimposing this on the RF which remains at a fixed frequency. Sweeping the RF/DC ratio identifies the discrete ratio corresponding to a specific mass, and an output is produced at the detector for each mass present. The height of each m/z peak corresponds to the relative abundance of each ion (Jones *et al.*, 1991).

The instrument is evacuated to 10^{-4} - 10^{-8} Pa to reduce ion loss through molecular collisions. Different vacuum systems include diffusion, turbomolecular and cryogenic pump systems. All insertions into the vacuum system must be via a vacuum lock, e.g. GC column exchange (Mitchum, 1991).

3.4.2.2 MSMS tandem spectrometry

The triple stage quadrupole mass spectrometer has more than one mass analyser, and can be used for MSMS analysis. This highly selective technique provides an additional degree of confirmation over single stage MS for complex samples containing co-extractives. A parent ion from a compound separated by the first analyser is fragmented in a collision cell into characteristic daughter ions, and can be mass separated on the second mass analyser (**Figure 3.4.2.2.1**).

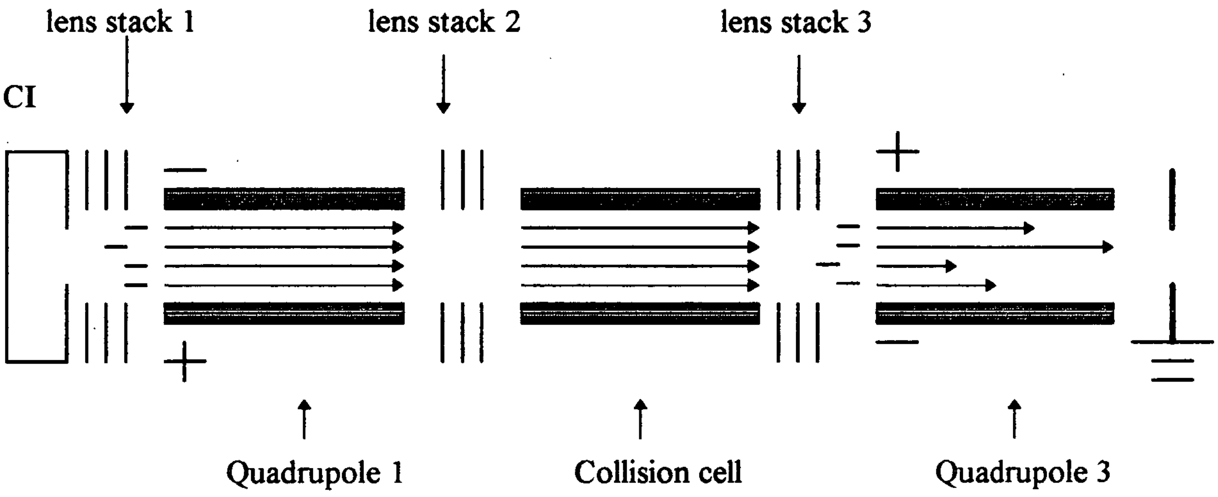


Figure 3.4.2.2.1 Schematic of the MSMS system.

3.4.2.3 Ionisation techniques

During Electron impact ionisation (EI), neutral sample molecules are bombarded by an electron beam from an ion source (heated filament), leading to the stripping (+ve ion) or adding (-ve ion) of an electron. High sensitivity is achieved at an average electron energy of 70eV. In Positive chemical ionisation (PCI), the sample reacts with ionised gas molecules (reactant ions) leaked into the ion source. Transfer of a proton or another +ve charged species from the reactant ion is observed as a less energetic process. Fragmentation is therefore reduced relative to EI. This may be advantageous for high mass ions but can reduce ionisation efficiency commensurate with loss in absolute sensitivity. In addition, reactant ions can produce a high chemical background noise.

Electron capture negative chemical ionisation (NCI) relies on a different ionisation process. An incident electron beam (as EI) loses energy by interaction with electrons and molecules to produce slower, thermal electrons. These electrons will ionise neutral molecules with vacant low energy orbitals. As such, the reagent gas acts as a moderator and the technique is simple and selective for species with high electron affinity e.g. halogens, double bonded compounds, S, P etc. The high electron capture rates can result in better sensitivity compared to EI, aided by reduction of chemical interference to low levels.

The alternate emission of positive and negative ions from the source allows complementary information to be obtained from the PCI and NCI modes. This ionisation mode is termed Pulsed positive ion negative ion CI. Ionisation techniques are destructive to the molecules of interest.

3.4.2.4 Multiple ion detection (MID)

The mass spectrometer is programmed to jump from one pre-selected mass to another without collecting any information between the masses. This increases sensitivity due to the longer time spent for data acquisition on the masses of interest than when the instrument is on full scan.

3.4.3 Method development

Individual halocarbon standards at approximately 10 µg/ml in low haloform pentane were prepared as Section 3.3.3 (**Table 3.4.3.1**) in addition to mixed calibration standards of 0.5, 0.05 and 0.025 µg/ml. Each mixed standard contained 0.2 µg/ml CH_3I as internal standard. Following SE-54 column installation into the Varian 3400 gas chromatograph, the following method development steps were carried out before real samples were analysed (**Table 3.4.3.2**).

Table 3.4.3.1 Individual standards in low haloform pentane (Rathburn).

Halocarbon	CH_3I	$CHCl_3$	$C_2H_3Cl_3$	CCl_4	C_2HCl_3	C_2Cl_4
Concentration (µg/ml)	7.00	11.34	6.56	10.20	8.52	6.88

Table 3.4.3.2 GC/MS Method development steps. Selected spectra are given in Appendix 7.

Step	Scheme	Procedure	Conditions		Results
			GC	MS	
1	EI / chromatography optimisation and t_R (mins) identification	Sequential injection of 10 μg/ml standards (0.5μl)	SE54 + 1m RG, He carrier @ 7.0psi. Oven 38°C 12 min, 6°C/min to 120°C, 25°C/min to 250°C for 5min. SPI On column injector : 38°C for 0.1min, 140°C/min to 250°C for 34min	EI full scan 30-204 sec, acquisition time after 2 sec.	Halocarbon t_R Major EI ions CH_3I 1.22 m/z **142** $CHCl_3$ 2.36 m/z 43,47,83,85,87,**118** $C_2H_3Cl_3$ 3.07 m/z 61,**97**,99,101,117,119,121 CCl_4 3.28 m/z 82,84,**117**,119,121 C_2HCl_3 4.31 m/z 95,97,**130**,132,134 C_2Cl_4 10.41 m/z 129,131,133,**164**,166,168,170 Designated parent set mass (PSM) in **bold**
2	ECNCI (CH_4) optimisation using standards	0.5 μg/ml mix + 0.2 μg/ml CH_3I (0.5μl)	Column as above, oven @ 38°C isothermal. Injector : 38°C for 0.1min, 140°C/min to 250°C for 10 min	(1) ECNCI (CH_4) Full scan. 8 x 10^{-6} torr (manifold), source 100°C	All (except C_2Cl_4) failed to give suitable spectra due to halogen ion detachment $R\text{-}X \rightarrow R\bullet + X\bullet^-$
				(2) PCI	Unsatisfactory

3	EI-MRM optimisation (MSMSC and collision offset (cooff)) for each chlorocarbon at 0.5,1.0,2.0 mtorr Ar collision gas	10µg/ml stds 1µl injection	Column as above 38°C isothermal oven. Injector as above.	(1) Full daughter scan. Ar collision gas 0.5,1.0, 2.0 m torr (pirani) MSMSC=70 (2) Ar 1.1x 10^{-5} torr. Ar 1-2 mtorr varied,pirani. MSMSC=30	Optimum conditions MSMSC cooff Ar(mt) Pkwth Reaction t_R CH_3I 70 -49 0.5 142→127 $CHCl_3$ 70 -14 2 8sec 118→83 2.60 $C_2H_3Cl_3$ 70 -49 2 10sec 97→61 3.12 CCl_4 70 -34 2 10sec 117→82 3.47 C_2HCl_3 70 -29 2 10sec 130→95 4.52 C_2Cl_4 70 -19 2 10sec 164→129 10.68 Further optimisation as Step 4. System re-tuned.
4	EI-MRM optimisation (alter MSMSC values for individual chlorinated solvents)	0.5 µg/ml mix + 0.2 µg/ml CH_3I (1.0µl injection)	SE54 (30m, 0.32mm ID, 1µm film) + 1m RG, He @ 7.0psi. Oven 38°C 12 min, 6°C/min to 120°C, 25°C/min to 250°C for 5min. SPI On column injector : 38°C for 0.1min, 140°C/min to 250°C for 23min. Xfer line 250°C	MIS scan to select for daughter ions. Ar 1.1 x 10^{-5} torr (manifold) MSMSC 30,50,50,50, 30 (elution order).	Data satisfactory. MSMS method as above with adjusted MSMSC used to run calibration standards, field blanks and samples. Refer to Appendix 7 for example calibration, field blank and sample data.

3.4.4 Results and discussion

The peak area data used to determine that the response of the GC/MS system is linear for the target compounds over the expected concentration range as N (time during a 24 hour autosampler run) increases are given in Appendix 7. During the autosampler run, retention time variability was acceptably low at ±0.5%.

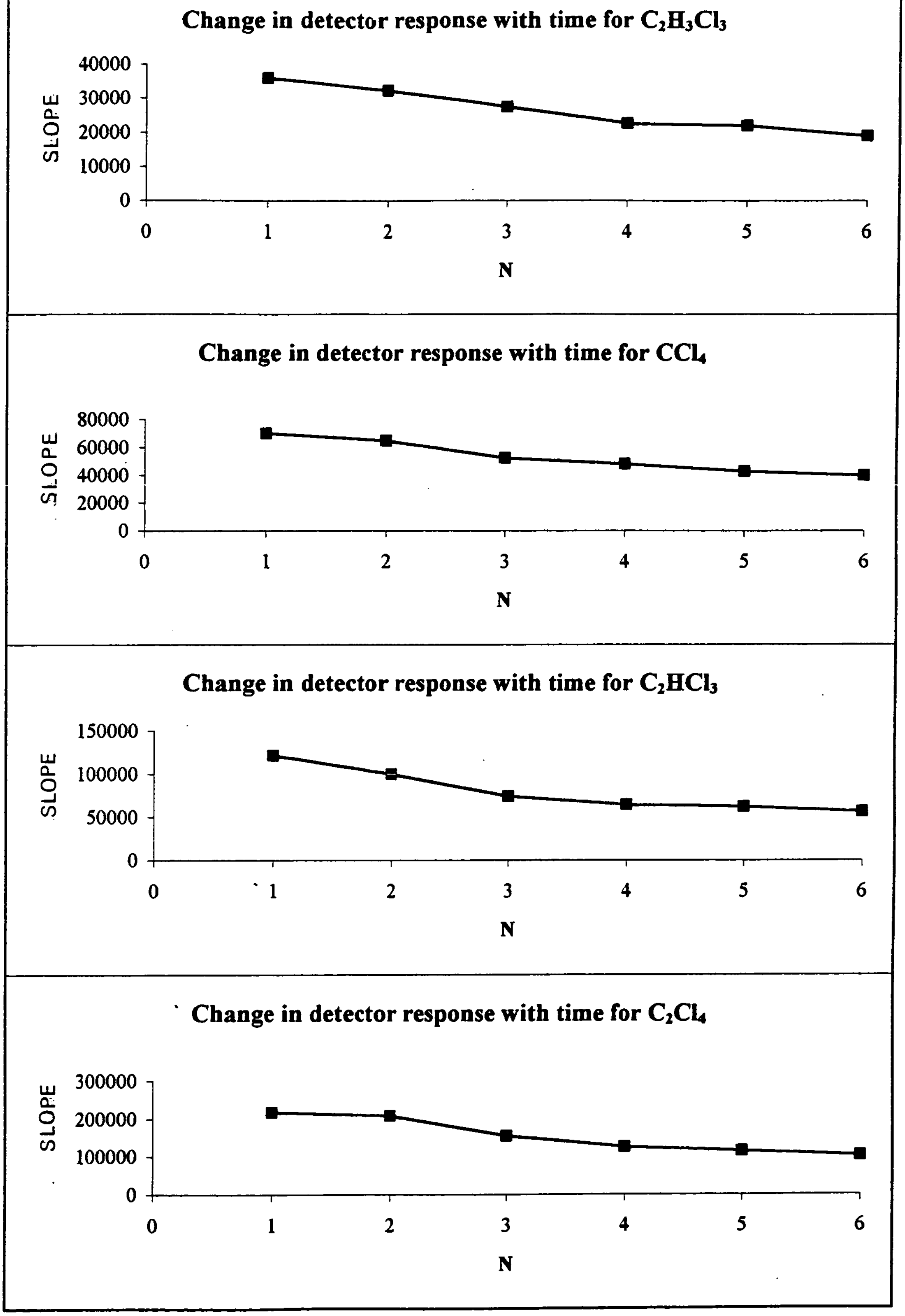
Although the calibration standards were analysed with a constant concentration of CH_3I present, artefact formation at such low concentrations prevented data collection for CH_3I and $CHCl_3$. Consequently, response factors relative to the internal standard similar to those obtained by GC/ECD could not be determined.

Response factors were generated using six calibration sets (0.025, 0.05, 0.5 μg/ml) analysed during the autosampler run over 24 hours. All the samples analysed were interspersed between the calibration sets. Plotting known concentration (x) Vs peak area count (y) as a best fit linear regression allowed the determination of the slope (response) and intercept for each calibration set (N). All the regression lines for all the compounds had intercepts that were not significantly different from zero, hence only the response (slope) values are given in **Table 3.4.4.1**. An example of a regression calculation for C_2HCl_3 is given in Appendix 7. The minimum detectable amount (**Table 3.4.4.1**) for each N was calculated from the 95% confidence limit on the slope.

Table 3.4.4.1 shows that there is a systematic decrease in response with increasing N. This is shown more clearly in **Figure 3.4.4.1**, and may have been due to column conditioning at the higher operating temperature. The response factor and intercept generated from the calibration data at given N are used when calculating concentrations in samples also analysed at that N. An example calculation for C_2HCl_3 concentrations in Devilla needles is given in Appendix 7. Note that a limit of detection (LOD) is generated for each calibration set. The LOD is defined as the intercept plus three times the standard deviation of *y* on *x* (Miller and Miller, 1994; Chapter 5).

Table 3.4.4.1 Slope and calculated LOD from GC/MSMS calibration plots.

Chlorinated solvent	N	Slope (response, area/µg)	Standard error on slope	95% confidence limit on slope	LOD (Minimum detectable amount, µg/ml)
$C_2H_3Cl_3$	1	35900	100	440	0.012
	2	32300	190	800	0.025
	3	27400	310	1400	0.049
	4	22500	250	1100	0.048
	5	22000	380	1600	0.073
	6	18900	73	310	0.017
CCl_4	1	69600	550	2400	0.034
	2	64600	830	3600	0.055
	3	52200	260	1100	0.022
	4	48000	360	1500	0.032
	5	42600	650	2800	0.065
	6	39900	330	1400	0.047
C_2HCl_3	1	121000	360	2100	0.016
	2	99200	760	3300	0.033
	3	73900	490	2100	0.029
	4	64000	360	1500	0.024
	5	61800	730	3200	0.051
	6	56200	630	2700	0.049
C_2Cl_4	1	216913	112	483	0.002
	2	207548	2129	9176	0.044
	3	154273	1125	4849	0.031
	4	124631	713	3073	0.025
	5	115111	357	1539	0.013
	6	102681	955	4116	0.040



Change in detector response with time for $C_2H_3Cl_3$

Change in detector response with time for CCl_4

Change in detector response with time for C_2HCl_3

Change in detector response with time for C_2Cl_4

Figure 3.4.4.1 Change in detector response with time. Note the different scales on the *y* axis.

Due to the limited time available it was not practical to carry out the full sequence of validation checks, including matrix spiking and determination of recovery to detect analytical variability. The results obtained can however be used to determine some approximate field blank-corrected chlorinated solvent concentrations (**Table 3.4.4.2**). Of primary interest, though, is the confirmation of chlorinated solvent presence in the leaf wax matrix, i.e. qualititative rather than quantitative information.

All the sample data obtained using GC/MSMS were below the formal LOD. This result is comparable to that obtained from GC/ECD analyses of pine needles. Confirmation of amounts of chlorocarbons present was not possible as not all the SASA quality control criteria were satisfied and no replicates were analysed. However, the exercise was useful in that some of the amounts determined in foliage were corroborated. The GCMS analyses also showed that there was no indication of any chlorocarbon presence at these detection limits in the Rathburn extraction pentane. The chlorinated solvent elution order was verified by the calibration standard data.

Table 3.4.4.2 Comparison of GCMS needle data with mean of 3 GC/ECD analyses. Samples were prepared identically for both techniques. ND = no peak detected.

Needle sample		**Chlorinated solvent (ng/g dry weight)**							
		$C_2H_3Cl_3$		CCl_4		C_2HCl_3		C_2Cl_4	
		GC/ECD	GC/MSMS	GC/ECD	GC/MSMS	GC/ECD	GC/MSMS	GC/ECD	GC/MSMS
LOD		30	25	7	52	310	64	7	35
Devilla	**13m 1992**	5±21	ND	3±1	ND	75±118	-0.81*	29±17	ND
21/04/94	**13m 1993**	18±27	ND	2±2	ND	131±89	20	29±20	ND
	10m 1992	8±24	ND	1±4	ND	-90±144	12	4±7	ND
	10m 1993	-1±8	ND	2±1	ND	146±149	6	5±9	ND
LOD		301	134	13	124	324	132	39	38
Devilla	**13m 1992**	101±173	ND	5±1	ND	227±59	ND	11±11	ND
10/02/94	**13m 1993**	-7±30	9†	10±12	-7*†	220±268	ND	21±22	ND
Frozen	**10m 1992**	12±9	ND	10±5	ND	104±155	ND	12±1	ND
	10m 1993	-38±9	ND	0.01±0.6	ND	90±65	ND	4±10	ND
LOD		20	127	8	40	277	113	15	37
Banchory	**1992**	33±17	ND	2±7	ND	163±101	ND	7±13	ND
24/01/94	**1993**	11±17	ND	-3±0	ND	21±56	ND	2±1	ND
Frozen									
LOD		113	119	33	113	485	280	91	91
Ponsonby	**W 1992**	27±19	ND	22±10	ND	439±362	ND	41±83	ND
16/02/94	**W 1993**	28±34	ND	5±3	ND	295±238	ND	50±60	ND
Frozen	**C 1992**	63±68	ND	23±42	ND	359±724	ND	81±44	ND
	C 1993	103±33	ND	69±51	ND	690±754	ND	66±80	ND
	E 1992	39±27	ND	14±3	ND	439±257	ND	54±6	ND
	E 1993	38±13	ND	31±6	ND	343±532	ND	8±8	ND

* Negative as field blank-corrected result. † Trace detected.

3.4.5 Conclusions

(1) Complementary qualitative information has been obtained which confirms the presence of the chlorinated solvents of interest in samples of Scots pine needles.

(2) In the limited time available, the GC/MSMS system could not be tuned to sufficiently low detection limits to allow quantitative determination of the amounts present in the needle samples.

(3) The technique has potential and should be investigated further. Reducing the lower limit of the calibration standards used would lower the effective detection limit.

Chapter 4

Partition ratio studies

‘So I turned my mind to understand, to investigate and to
search out wisdom and the scheme of things’
Ecclesiastes

4 PARTITION RATIO STUDIES

4.1 Introduction

The experimental determination of chemical equilibria between whole plants and plant organs and air is important for several reasons. The air to leaf transfer is a key pathway for uptake of organic compounds into plants, and several sorption compartments can be recognised based on uptake and accumulation studies (Paterson *et al.*, 1990; Schreiber and Schönherr, 1993).

Leaf / air bioconcentration factors (BCF) ($ng.g^{-1}$ leaf/$ng.g^{-1}$ air) and partition ratios (gm^{-3}/gm^{-3}) can be used to develop a correlation with K_{ow} (octanol/water partition coefficient) as a measure of lipophilicity and H (Henry's law constant, $Pa.m^3/mol$ (Bacci *et al.*, 1990)) reflecting water solubility (**Table 4.1.1**). The limitations of K_{ow} and H as physical constants which do not depend on plant properties must be recognised. For example, the rate of attainment of equilibrium, distribution of compounds in different sorption compartments, and dissipation by metabolism/revolatilization depend largely on plant properties. All are of toxicological significance. The only plant properties that enter into the BCF are the total size and capacity of the sorption compartments (Schreiber and Schönherr, 1993). K_{aw}, the dimensionless air/water coefficient, is also related to H ($K_{aw} = H/RT$) where R is the gas constant ($8.314\ Pa.m^3/(mol.K)$) and T is the temperature (K) (Bacci *et al.*, 1990).

In order to understand the relationship between pine needle concentrations of chlorinated solvents and ambient concentrations in air, a knowledge of the partition coefficient is required.

Table 4.1.1 Selected physiochemical properties for the chlorinated solvents of interest. Data for molecular weight, Henry's law constant, *H* (20°C), log K_{ow} (octanol-water partition coefficient) and solubility (20°C) from ECETOC technical report, 1988. Data for vapour pressure (20°C) from Mackay, 1991.

Chlorinated solvent	Molecular weight	Vapour pressure, Pa	H, Pa.m^3/mol	log K_{ow}	log K_{aw}	Solubility in water, g/m^3
$CHCl_3$	119.38	20000		1.97[a]		8200[b]
$C_2H_3Cl_3$	133.41	13000	13000	2.95	0.727	130
CCl_4	153.82	12000	1530	2.64[c]	-0.202	1200
C_2HCl_3	131.39	9870	920	2.53[d]	-0.423	1400
C_2Cl_4	165.83	2100	730	3.30[e]	-0.523	480

[a] From Mackay 1991. Value of 2.00 from Bunce, 1991; [b] from Mackay, 1991; [c] From Bunce, 1991, Value of 2.70 from ECETOC, 1988; [d] Value of 2.47 from Bunce, 1991. Value of 2.29 from Mackay, 1991 ;[e] Value of 2.60 from Bunce, 1991.

A standard atmosphere generator with exposure chamber was designed and built to determine the partition ratio between Scots pine needles or needle wax extract and air for $C_2H_3Cl_3$, CCl_4, C_2HCl_3 and C_2Cl_4. Wax and needle concentrations of the chlorinated solvents were determined using optimised GC/ECD as before (Chapters 2 and 3). $CHCl_3$ was excluded due to interference from high laboratory background levels (refer to Chapter 5).

4.2 Method

A method was developed to:

(1) Determine the time (t_e) taken for an equilibrium to be established at 20°C between chlorocarbon concentration in Scots pine needle wax or whole needles and air;

(2) Determine (at t_e) the variation of chlorocarbon concentration in Scots pine needle wax extract or whole needles with increasing air concentration leading to calculation of partition ratios.

4.2.1 Experimental design

The apparatus used to generate individual chlorinated solvent atmospheres of known concentration, and to expose whole needles or wax supported on discs to these atmospheres is shown in **Figure 4.2.1.1**. Control of 99.995% purity cylinder N_2 flow (simulated air) was by needle and on/off valves. All piping (3.2mm diameter) was cleaned copper (Perkin Elmer), and pipe-to-glass tube fittings were O-ring sealed brass. Source and exposure chamber seals were glass quickfit (Bibby Sterilin Ltd.) as was the 75ml source tube. The solvent vials were 5ml glass (Sigma Aldrich), sealed with plastic, holed caps with PTFE membranes to permit loss of solvent vapour into the source tube. Pure solvents used were as supplied by Sigma Aldrich. The exposure chamber was a 25cm diameter glass dessicator with ground glass and rubber O-ring seal.

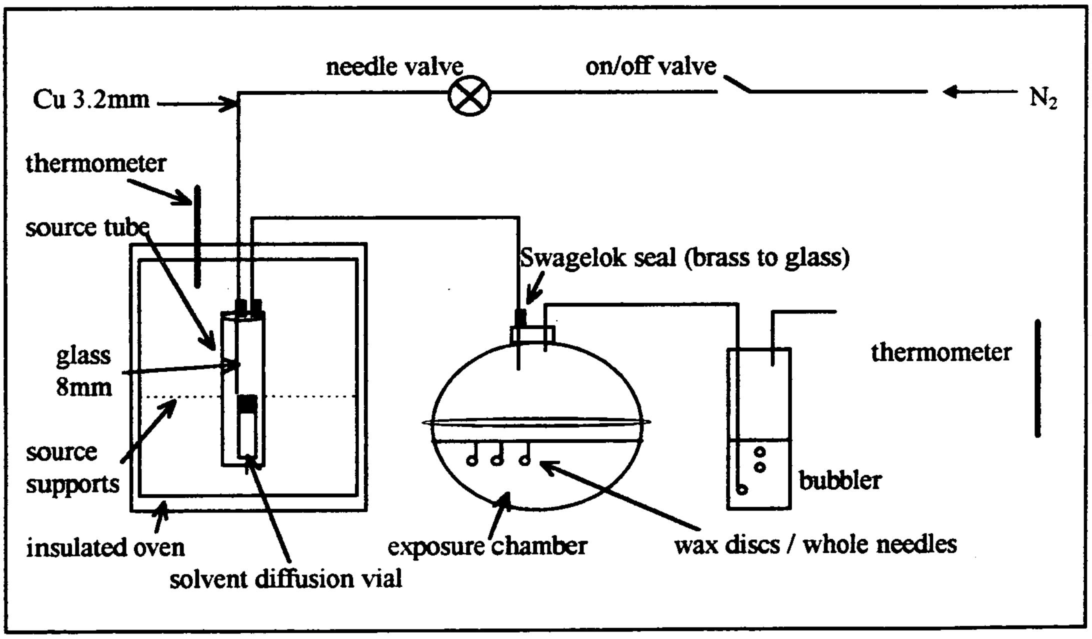


Figure 4.2.1.1 Experimental design for partition ratio studies. The exposure chamber was at room temperature.

Individual stainless steel mesh discs (10mm diameter) held on hooks, were used to support the wax extract in the exposure chamber. Whole needles were supported in a copper wire basket. Venting of the exposure chamber was via plastic tubing to a bubbler containing pentane, to trap chlorinated solvent waste.

4.2.2 Method development

The chlorinated solvent loading (gm^{-3}) of the simulated air was determined from the N_2 flow rate (ml/min) and the mass difference (g) of the pure solvent in the diffusion vial over a 24 hour period. Control was primarily through oven temperature, and to a lesser extent, the hole size of the vial cap and number of PTFE membranes used. The wax coating on the stainless steel discs was prepared as described in Chapter 2. The coated discs (or Scots pine needles collected from 2m height, 1992 year class, Kings Buildings, Edinburgh) were exposed at room temperature to a continuous flow of standard atmosphere over a range of times before extraction into pentane and GC analysis as in Chapter 3.

The chlorinated solvent detection limits of the analytical technique dictated the amount of wax on the discs and the final gas phase concentration to ensure retention of a sufficient amount for analysis. Based on >10 pg/μl chlorinated solvent detection limit in pentane after extraction and an approximate concentration of 0.1g wax/ml pentane, a concentration of 100 ng of chlorinated solvent per g of wax was needed. At a partition ratio of 100 ($g.m^{-3}$ wax /$g.m^{-3}$ air), a concentration of > 1μg/L (mg/m^3) air was needed, implying a diffusion rate of >0.5μg/min into an N_2 flow of 0.5 L/min, or a diffusion rate of 720 μg chlorocarbon per day. Using a more practical concentration of wax in pentane of 1mg wax/ml, concentrations of >100 mg/m^3 air were required. An N_2 flow of 0.5 L/min with appropriate manipulation of oven temperature between 50 and 80 °C provided a flow of 5-350 µg/min from the solvent source corresponding to 10-700 mg/m^3 air concentrations and leading to 7.2-504 mg/ day solvent loss. As the actual detection limit in many cases was >10 pg/injection and the anticipated partition ratios were of the order of 400-2200 (Frank and Frank, 1986), these values were acceptable.

Molten wax was coated onto the discs (0.8mg per disc) using clean, heated (to prevent wax solidification) glass pipettes under fumehood conditions. Each needle sample was approximately 1g fresh weight. Non-exposed wax discs and needle blanks were made up and analysed identically. Pentane (Rathburn, low haloform) extraction volumes were 1ml (discs) and 2ml (needles). Needles were extracted for 24 hours at 4°C prior to GC/ECD (Chapter 2) and discs for 1 hour at 20°C. The method used to establish t_e and the partition ratios was as follows;

(1) GC/ECD validation standard run(s).
(2) Set oven temperature, N_2 as purge at 0.5 L/min (bubble flow meter).
(3) Weigh pre-cleaned and oven-dried (100°C for 24 hours) source vial.
(4) Re-weigh containing 2ml of one pure chlorinated solvent.
(5) Place in source tube in heated oven. Note time.
(6) Check N_2 flow of 0.5 L/min at exposure chamber.
(7) †Weigh discs, coat with wax and re-weigh.
(8) Hook up discs in exposure chamber. Check N_2 flow. Note time, room temperature.
(9) Expose discs for appropriate time. Run blanks on GC/ECD (N=3).
(10) Remove discs, extract in pentane for 1 hour, seal with Teflon-coated septum.
(11) Run on GC/ECD (N=3). Note time, room temperature.
(12) After 24 hours, remove source vial, allow to cool and re-weigh. Note time.

† about 1g (vial batch) of fresh needles were weighed for the needle experiments.

The time to reach equilibrium, t_e, was established by exposing each of a batch of identical wax discs or needle batches collected at the same time to a known chlorinated solvent concentration on an approximately logarithmic time basis (1,2,4,6,8,24 hours). Method objective 2 was realised by exposing discs or needles to varying chlorinated solvent concentrations for times longer than t_e on separate days.

4.2.3 Validation of method

Multiple exposures (separate discs or needle samples) at particular concentrations showed that results were consistent to within ± 20% for wax and ±5% for needles (**Table 4.2.3.1**).

Table 4.2.3.1 Reproducibility data for the partition ratio method. The data given are replicate discs from the same experiment except for $C_2H_3Cl_3$ when two separate experiments were run. Triplicate GC/ECD analyses were made of the extraction solvent for each replicate disc or needle batch (approx. 1g). The mean and standard deviation values of the replicate discs are in bold. Conversion from pg/μl to g/m^3 uses the experimentally determined wax and needle density of 0.82g wax per cm^3 and 0.89g fresh weight per cm^3 respectively (refer to Appendices 2, 6 and 8).

Chlorinated solvent	Concentration in air (gm^{-3})	Concentration in wax (gm^{-3} wax)	Concentration in air (gm^{-3})	Concentration in whole needles (gm^{-3} fresh weight)
$C_2H_3Cl_3$	(1) 0.072	19, 21,26,32 **25±6**	0.002	0.03, 0.03,0.03 **0.03±0.0003**
	(2) 0.072	28, 26, 27 **27±1**		
	0.311	169, 172 **171±2**		
CCl_4	0.0168	1.0, 1.4 **1.2±0.3**	0.318	8.9,9.1,9.2 **9.1±0.1**
	0.633	53, 62,64 **60±6**		
C_2HCl_3	0.019	0.9, 1.8 **1.33±0.62**	0.148	8,9,9 **9±0.1**
C_2Cl_4	0.019	27, 27, 27 **27±0.4**	0.019	0.5,0.5,0.5 **0.5±0.004**

4.3 Results and discussion

4.3.1 Detection limits

The detection limits and blank levels determined are in **Table 4.3.1.1**. These levels are higher than those obtained for air and needle field samples. Whole needles blank levels in particular were subject to wide variation as reflected in the high detection limits. Lower concentrations of standard atmosphere were used first to minimise the possible effects of carry-over of small quantities of chlorinated solvent.

Table 4.3.1.1 Blank levels and detection limits for the partition ratio method.

Chlorinated solvent	wax discs		whole needles	
	Blank (pg/µl)	Detection limit ($2sd_B$)	Blank (pg/µl)	Detection limit ($2sd_B$)
$C_2H_3Cl_3$	30±15	30	90±18	36
CCl_4	2.44±0.32	0.64	41±49	98
C_2HCl_3	5.19±2.17	4.34	14±32	64
C_2Cl_4	71±52	104	60±25	50

The analytical results in pg/µl were corrected by subtraction of the blank and converted to $g.m^{-3}$ using the experimentally determined wax density ($0.82gcm^{-3}$, Appendix 2) and needle density ($0.89gcm^{-3}$, Appendix 6). These were used to determine partition ratios as (gm^{-3} wax or needle)/(gm^{-3} air) (Appendix 8). An example calculation of the BCF is also included for comparison in Appendix 8. The correction for the blank values is based on calculation of the mass of solvent attributable to the blank (q_B), and subtraction of this from the total mass of solvent determined (q_S) to produce q_{cor}. So :

$$\frac{mass\ of\ solvent\ for\ blank}{mass\ of\ wax\ for\ blank} = \frac{q\ (ng)}{m_B\ (mg)}$$

The mass of sample (m_s) is known, and the mass of solvent attributable to the blank

is q_B where $$q_B = \frac{q}{m_B}.m_s$$

leading to $$q_{cor} = q_S - q_B.$$

4.3.2 Equilibrium studies

The uptake of the chlorinated solvents by both wax and Scots pine needles is fast, reflecting their lipophilic character and the permeability of the cuticle (Sato and Nakajima, 1979). Attainment of an equilibrium with air was rapid over the standard atmosphere concentration range used. The values obtained for t_e are summarised in **Table 4.3.2.1**. Literature values for beeswax and spruce needles (*Picea abies*) are included for comparison (Frank and Frank, 1986). **Table 4.3.2.2** gives the data used to obtain the t_e values, and **Figures 4.3.2.1-4.3.2.4** show the time curves plotted.

Table 4.3.2.1 Values for t_e (hours) for the partitioning of chlorinated solvents between air and different lipid compartments. The initial air concentrations (gm^{-3}) are given in the table. Literature values are included for comparison.

	Scots pine wax discs		Beeswax model†		Scots pine whole needles		Spruce whole needles†	
	air conc^n	t_e	air conc^n	t_e	air conc^n	t_e	air conc^n	t_e
$C_2H_3Cl_3$	0.072	6	-	-	0.002	2	-	-
CCl_4	0.132	4	2.9	1.5	0.318	4	2.9	1
C_2HCl_3	0.049	2	2.8	5	0.148	6	2.8	4
C_2Cl_4	0.019	6	3.1	10	0.019	6	3.1	5

† From Frank and Frank, 1986. In both cases a 2L round bottomed flask was used; the inner surface of the flask was coated with beeswax; for the experiments with spruce needles amounts (25g of fresh needles) with similar surface areas (800cm^2) were used.

Table 4.3.2.2 Data used to obtain the equilibrium plots in Figure 4.3.2.1-4.3.2.4. Standard atmosphere concentrations are given in Table 4.3.2.1.

Chlorinated solvent	Time (hours)	Concentration in wax (gm^{-3})	Concentration in needles (gm^{-3})
$C_2H_3Cl_3$	1	72.63	0.0037
	2	40.17	0.0215
	3	26.49	
	4	31.67	0.0242
	6	27.52	0.0252
	8	25.80	0.0256
	21	26.56	
	23		0.0258
CCl_4	1	88.93	7.21
	2	38.22	7.75
	4	7.83	
	6	6.32	8.89
	8	5.89	9.12
	24	5.74	9.15
C_2HCl_3	1	3.74	1.78
	2	4.55	3.68
	4	5.62	5.63
	6	5.74	7.94
	8	5.80	9.13
	20	5.81	9.38
C_2Cl_4	2	50.02	0.083
	4	35.93	0.212
	6	26.68	0.481
	8	27.01	0.489
	20	27.43	
	22		0.483

The time dependence of the partitioning of chlorinated solvents between the atmospheric and lipid compartments is shown overleaf in **Figures 4.3.2.1-4.3.2.4** . Wax/lipid extract is given by open symbols, whole pine needles by filled symbols. The air concentrations used for t_e determination are also given in **Table 4.3.2.1**.

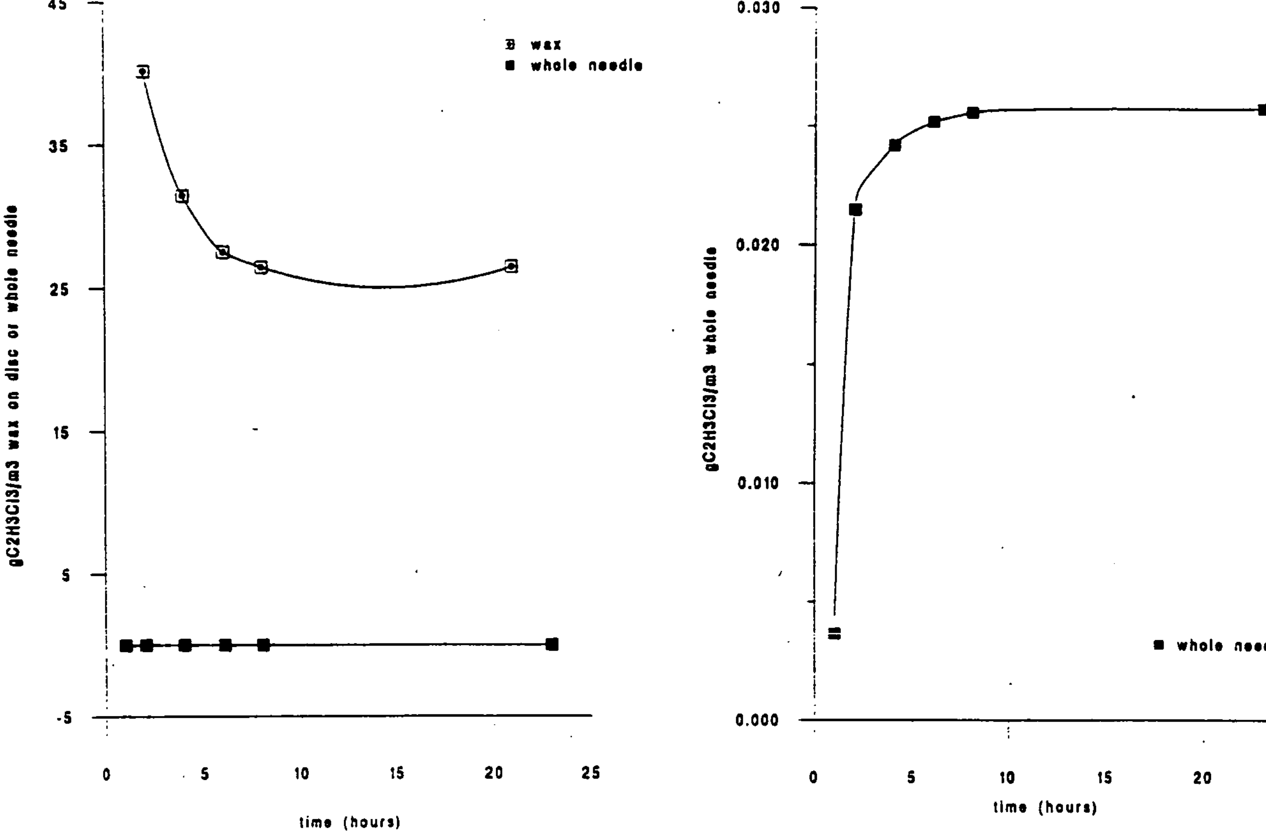


Figure 4.3.2.1 Time dependence of the partitioning of $C_2H_3Cl_3$ between the atmospheric and lipid compartments.

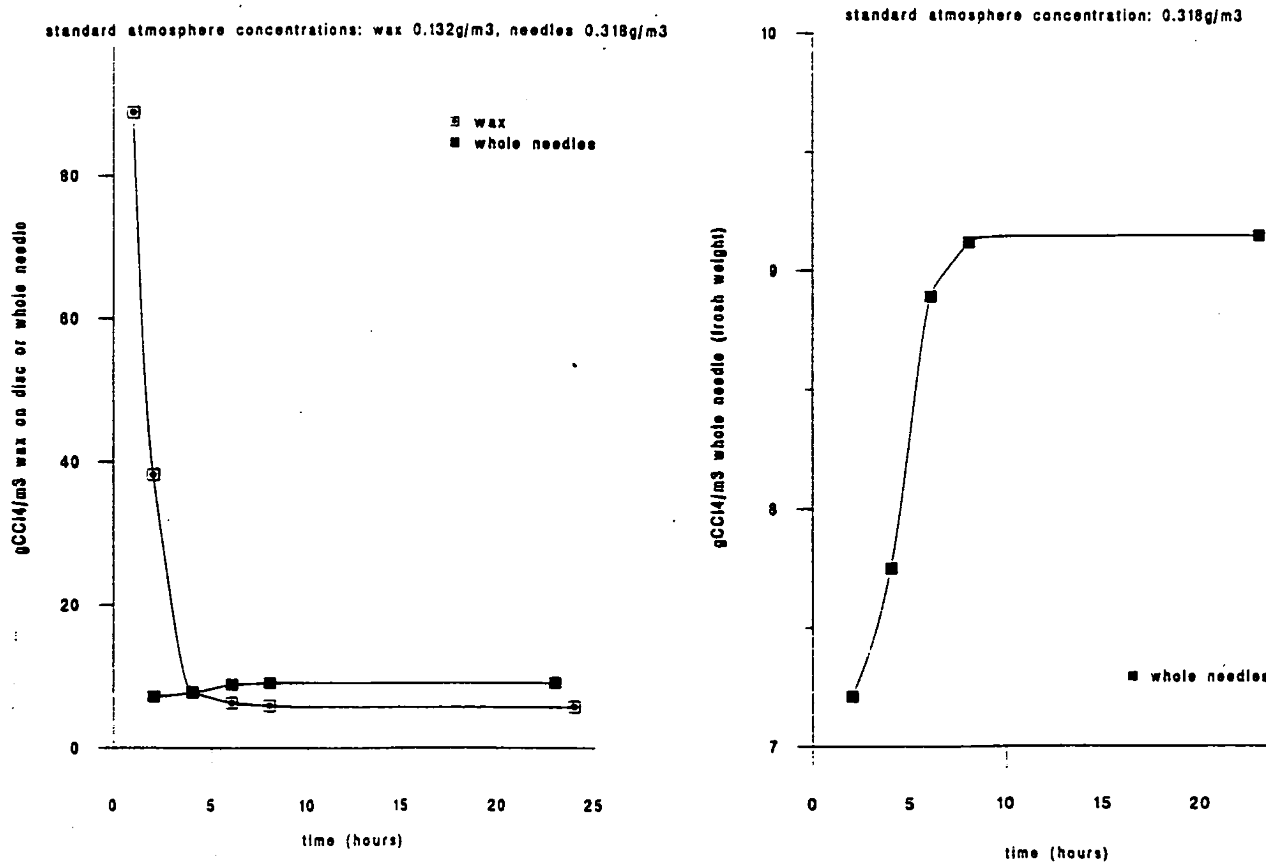


Figure 4.3.2.2 Time dependence of the partitioning of CCl_4 between the atmospheric and lipid compartments.

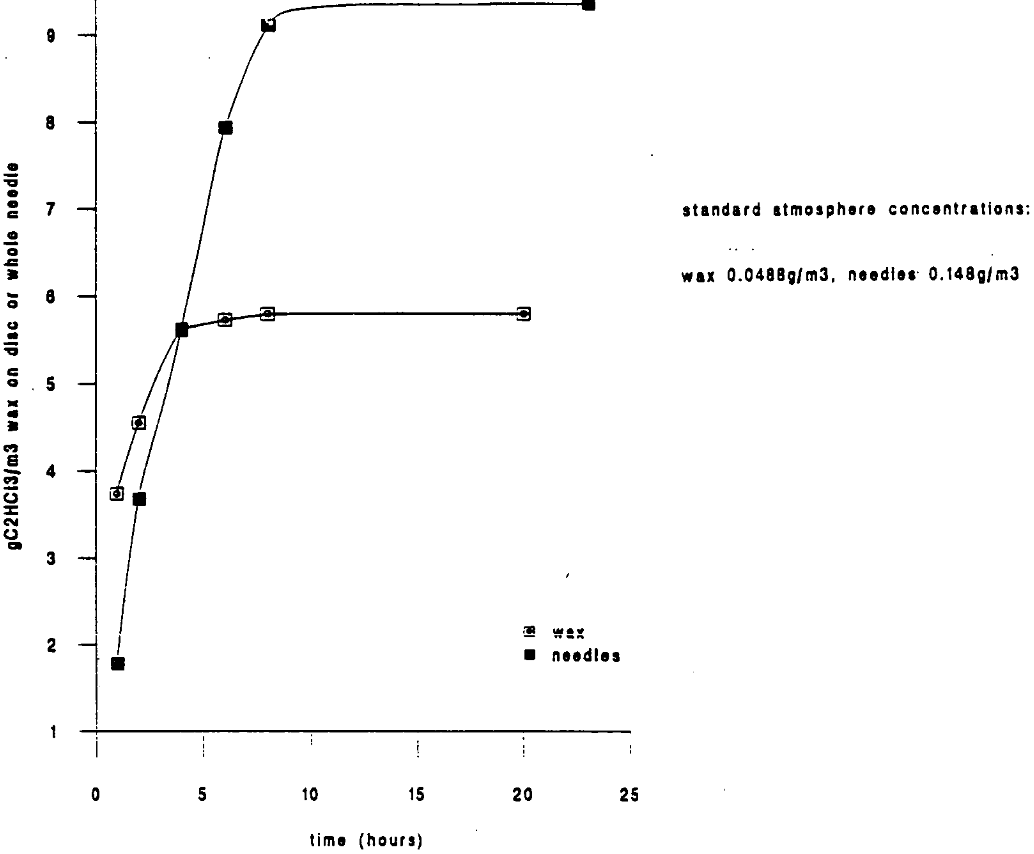


Figure 4.3.2.3 Time dependence of the partitioning of C_2HCl_3 between the atmospheric and lipid compartments.

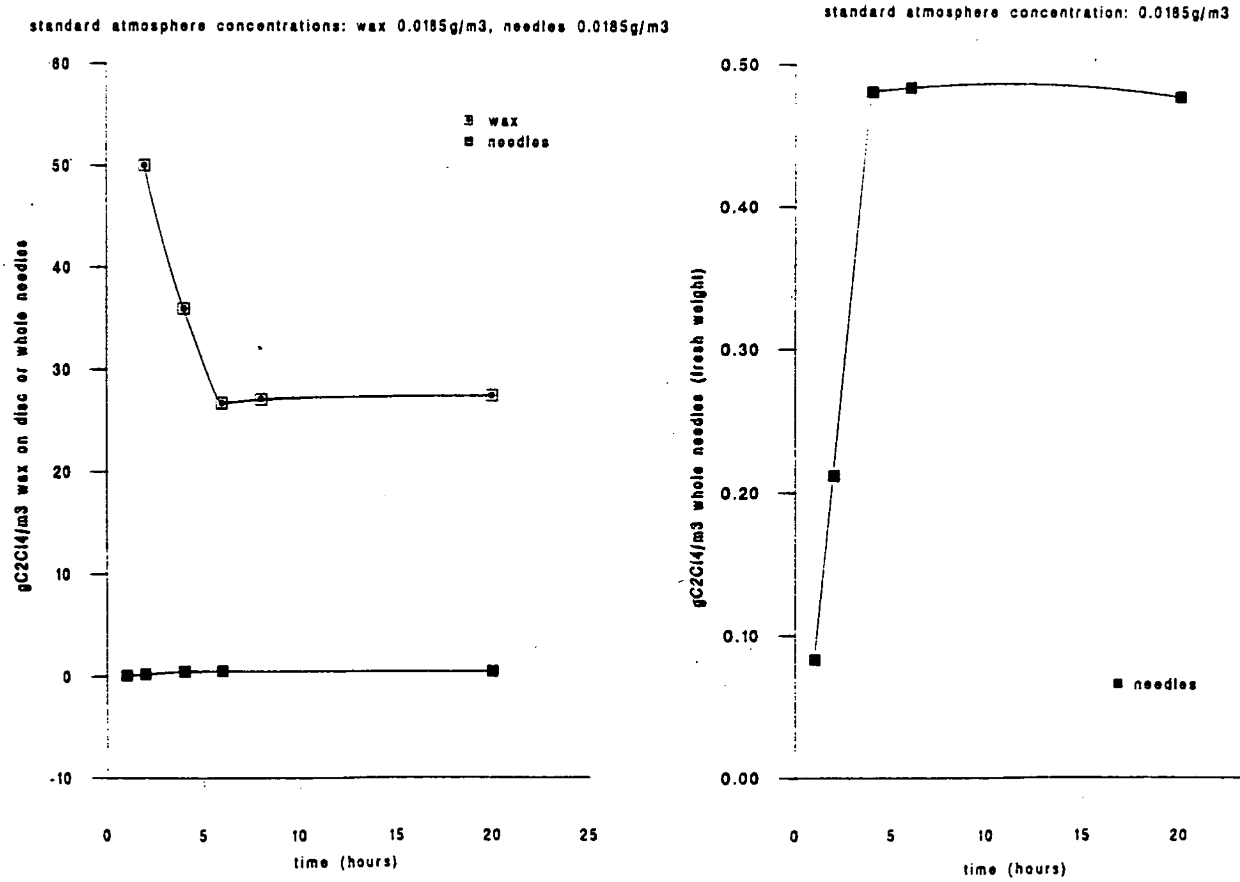


Figure 4.3.2.4 Time dependence of the partitioning of C_2Cl_4 between the atmospheric and lipid compartments.

The wax discs and the whole needles show different uptake behaviour. For three of the chlorinated solvents studied, $C_2H_3Cl_3$, CCl_4 and C_2Cl_4, the amount of chlorinated solvent in the wax decreases to equilibrium, while that for the whole needles increases to t_e. For C_2HCl_3, however, an increase to equilibrium for both the wax and the whole needles is seen. The experiments with beeswax and spruce needles by Frank and Frank (1986) suggest that an increase to equilibrium would be expected, as the concentration in the wax or needle increases to a maximum for the sorption compartment and then remains at this concentration in a saturated state. Thus the pine needles show the expected behaviour while that of the wax on the discs, in general, is different from that expected.

Several factors could account for this discrepancy. The beeswax previously used in model experiments may be less representative of the real situation in the leaf wax than a lipid/wax extract of the leaf wax itself. Alternatively, the wax discs could have been contaminated during extraction of the wax or preparation of the discs. Another possibility is that the method of generating the standard atmosphere may result in uptake anomalies which have no physical or chemical significance. For example, before equilibrium is achieved in the source, higher initial vaporisation rates may occur. After initial saturation of the septum in the source vial, which causes swelling, the diffusion rate may fall to the equilibrium value giving initial large concentrations before equilibrium is achieved. Whatever the explanation, particularly considering the inconsistency in uptake of C_2HCl_3 by the wax discs as compared to the other chlorinated solvents studied, the important fact is that an equilibrium distribution is established, and at times comparable to those found previously in the spruce needle study by Frank.

For C_2HCl_3 the initial (increasing) slope of the curve for Scots pine needles is four times greater than that of the wax, which is similar to the five times greater uptake rate found by Frank for CCl_4, C_2HCl_3 and C_2Cl_4. The slope of the curve (increasing) into the Scots pine needles for the other chlorinated solvents in this study is steeper before one hour than the slope (decreasing) for the wax discs, indicating that the rate of uptake into the needles is faster than the rate of loss of solvent from the wax as equilibrium is approached. In all cases, the establishment of equilibrium by t=6 hours allowed the partition ratio studies to be carried out.

4.3.3 Partition ratio studies

The chlorinated solvent partition ratios (gm^{-3}/gm^{-3}) between the lipid compartment and air were determined at t_e=6 hours for both the wax/lipid extract and the whole needles over an air concentration range of 2.5-1480 mg/m^3. These partition ratios are the slopes of linear plots of (g chlorocarbon per m^3) air Vs (g chlorinated solvent per m^3 wax on disc) (**Figure 4.3.3.1**) or whole needle (**Figure 4.3.3.2**). The data used is in **Table 4.3.3.1** and the correlation was good as is reflected in the high values of the correlation coefficient (r^2) (**Table 4.3.3.2**).

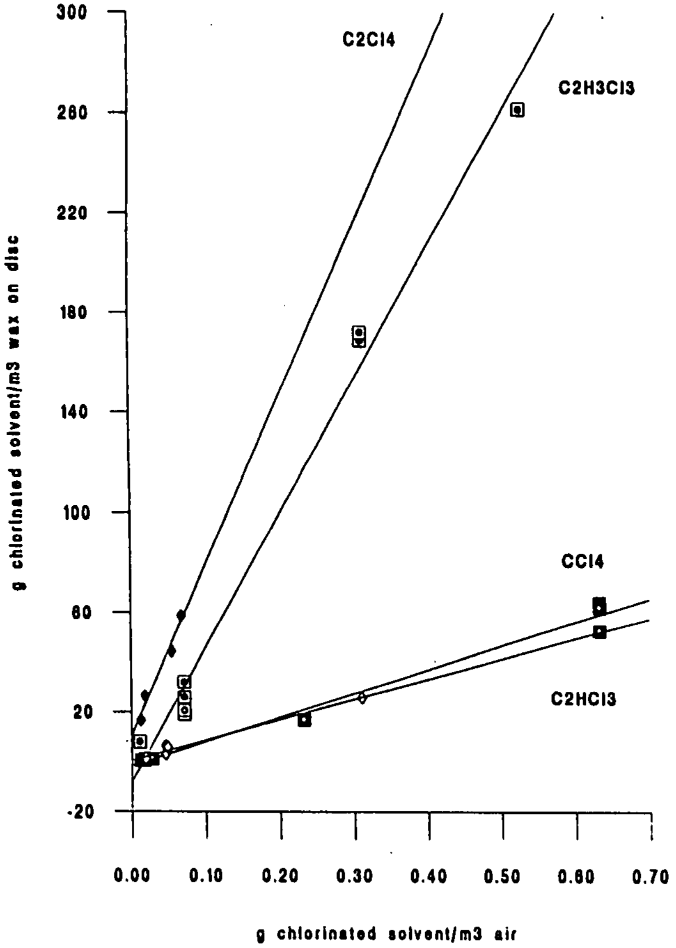


Figure 4.3.3.1 Correlation of standard atmosphere chlorinated solvent levels and the amounts extracted from a lipid/wax extract of Scots pine needles at 21°C. For some concentrations more than one disc was exposed to provide replicate data. Each point is the mean of three replicate analyses per disc.

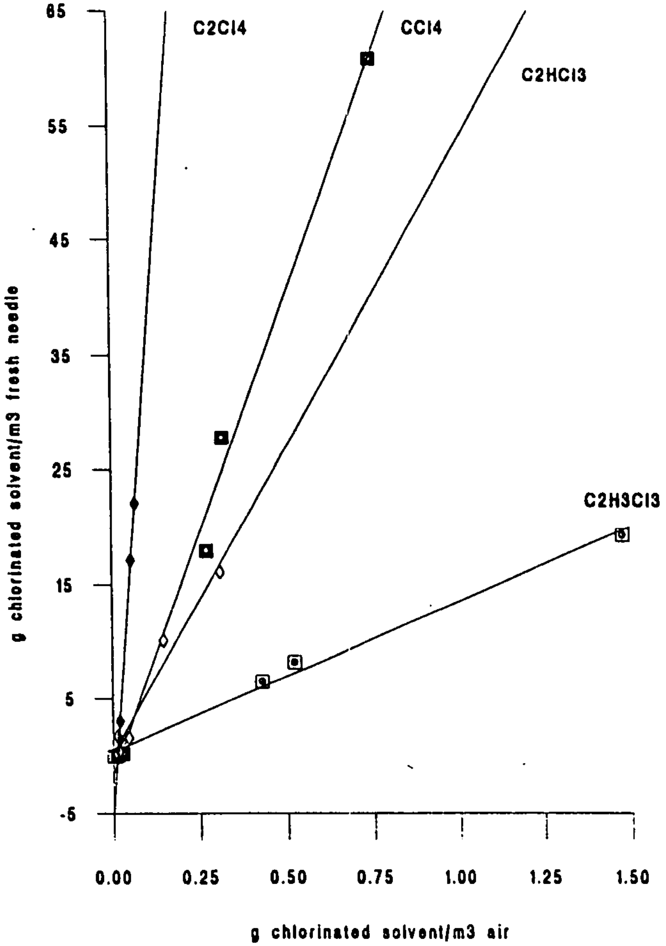


Figure 4.3.3.2 Correlation of standard atmosphere chlorinated solvent levels and the amounts extracted from Scots pine needles (1992 year class collected from a height of 2m) at 21°C. All GC/ECD analyses were performed in triplicate.

Table 4.3.3.1 Standard atmosphere wax extract and needle data (gm^{-3}) used to generate partition ratio plots. For some air concentrations, replicate discs were analysed. Values are means of triplicate GC/ECD analyses.

Chlorinated solvent	Air conc^n (gm^{-3})	Wax extract concentration	Air conc^n (gm^{-3})	Whole needle concentration
$C_2H_3Cl_3$	0.011	8.20	0.002	0.017
	0.072	19.12	0.007	0.039
		20.48	0.428	6.49
		25.84	0.521	8.22
		32.08	1.477	19.24
	0.312	169.02		
		172.01		
	0.528	261.66		
CCl_4	0.012	0.985	0.0124	0.105
	0.017	0.968	0.0274	0.283
		1.443	0.271	17.88
	0.027	1.507	0.318	27.75
	0.232	16.89	0.748	60.81
	0.633	52.69		
		62.21		
		64.28		
C_2HCl_3	0.0187	0.889	0.0095	0.391
		1.760	0.0187	1.520
	0.0460	3.447	0.0460	1.638
		6.523	0.148	10.08
	0.0488	5.934	0.311	16.05
	0.311	25.80		
C_2Cl_4	0.0126	16.79	0.0126	1.818
	0.0185	26.68	0.0185	0.463
	0.0549	44.31	0.0210	3.071
	0.0680	58.79	0.0549	17.09
			0.0680	22.11

Table 4.3.3.2 Coefficients from partition ratio plots, where the slope corresponds to the partition ratio. The errors given are the standard error on the slope and intercept respectively. None of the intercepts are significant at the 95% level.

Chlorinated solvent	Scots pine wax extract			Scots pine whole needles (fresh weight basis)		
	slope	intercept	r^2	slope	intercept	r^2
$C_2H_3Cl_3$	530±24	-8±6	0.988	13±1	0.5±0.5	0.992
CCl_4	96±5	-1±2	0.984	84±4	-1.7±1.6	0.993
C_2HCl_3	81±6	0.7±0.7	0.979	53±5	0.3±0.8	0.970
C_2Cl_4	680±90	11±4	0.967	400±30	-5±1	0.982

These results are similar to those of other authors (**Table 4.3.3.3**). The rapid partitioning observed in the needles represents sorption onto and into the cuticle (Schreiber and Schönherr, 1993) or uptake into the mesophyll membranes (Frank and Frank, 1986).

Table 4.3.3.3 Partition ratios of chlorinated solvents between lipid and air (21°C). Needle data are on fresh weight basis.

Chlorinated solvent	Scots pine lipid/wax extract	Total wax & lipid of Spruce[†]	Beeswax[†]	Scots pine needles	Spruce needles[†]
$C_2H_3Cl_3$	500	-	-	13	-
CCl_4	100	400	140	84	17
C_2HCl_3	80	1000	270	53	44
C_2Cl_4	700	2200	740	399	95[‡]

[†] From Frank and Frank, 1986. Temperature 22°C. Total lipid/wax (4.5% of fresh weight) extracted with cyclohexane and chloroform/methanol (2:1, v/v). [‡] Value of 129 from Figge, 1988.

The needles show a smaller partition ratio than the wax extract (**Figure 4.3.3.3**). Frank and Frank (1989) reported that two different linear correlations were obtained when concentrations of airborne C_2Cl_4 in fresh 1 year old spruce needles were plotted Vs concentrations in air; one at up to 5μg/m^3 and one above 10μg/m^3 (refer to Chapter 7). This indicated that two partition ratios existed, one of 520 in the lower concentration range (r^2=0.976) and one of 50 in the higher range (r^2=0.981). The existence of two compartments was suggested, a small, easily saturated one of high affinity (PR=520) possibly reflecting adsorption on the needle surface, and a larger one with a partition ratio of 50. It was concluded that in the concentration range common in clean air regions, small differences in actual air concentration translate into large differences in the dose taken up by the tree - lending support to the hypothesis that chlorocarbon uptake may contribute to stress in forest trees (see Chapter 8).

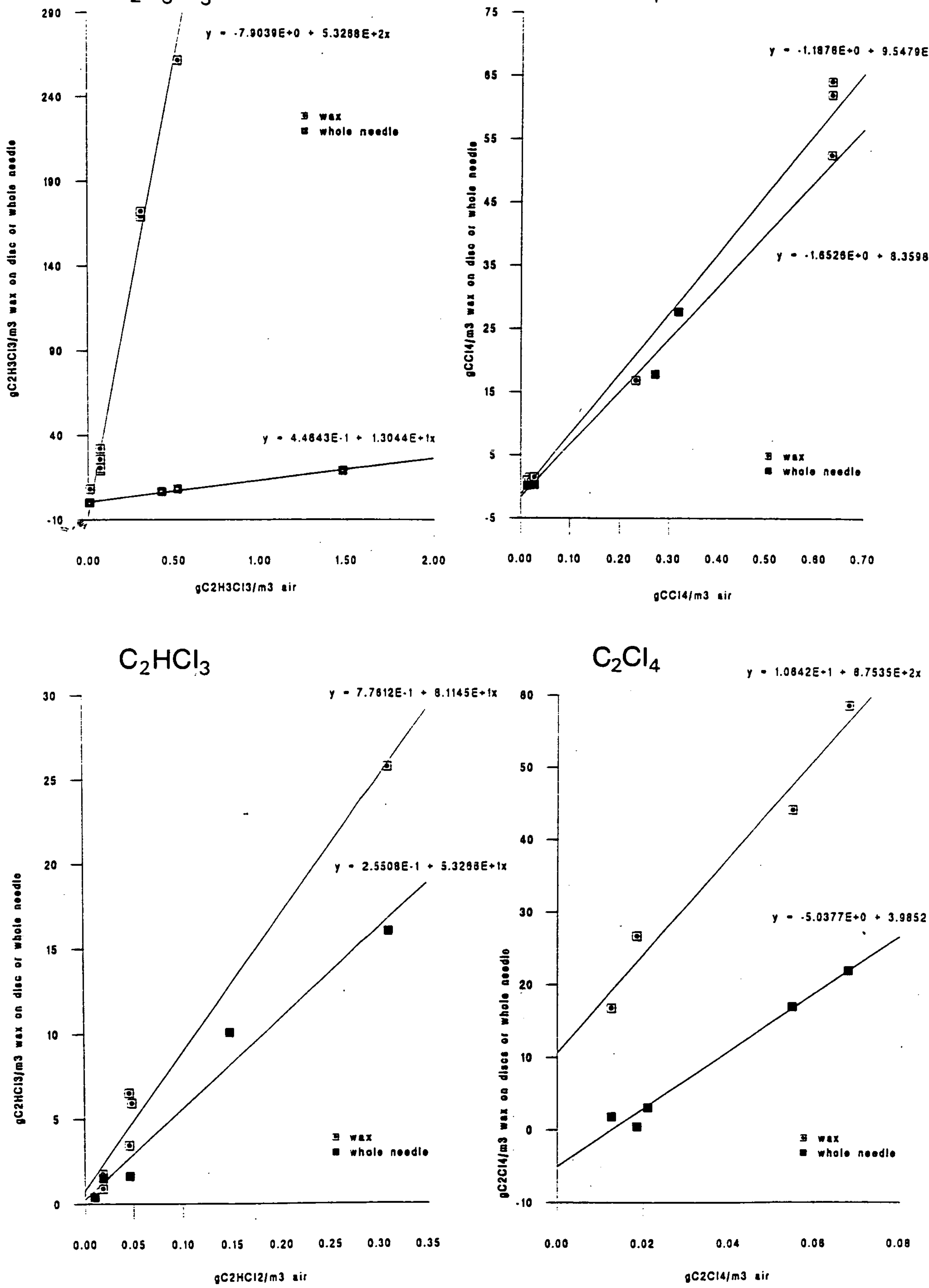


Figure 4.3.3.3 Comparison of chlorinated solvent uptake characteristics into Scots pine needle wax extract and whole needles at 21°C.

These types of detailed mechanistic deductions in whole needles are, however, not possible from the higher air concentration (2.5-1500 mg/m^3) data used in this partition ratio study (Chapter 7). Confirmation of the correct assignment of these compartments requires desorption kinetics studies of the chlorinated solvents from conifer needles (Schreiber and Schönherr, 1993).

4.4 Correlation of chlorinated solvent partition ratio with K_{ow}, H and K_{aw}.

The octanol/water coefficient, K_{ow}, has been used to indicate the affinity to lipids of different chemicals (Bacci *et al.*, 1990). The chlorocarbon wax:air partition ratio was found to increase with K_{ow}, **Figure 4.4.1** shows the log/log positive linear correlation (r^2 = 0.883). However, no such linear regression fit (log/log) was observed for the needles.

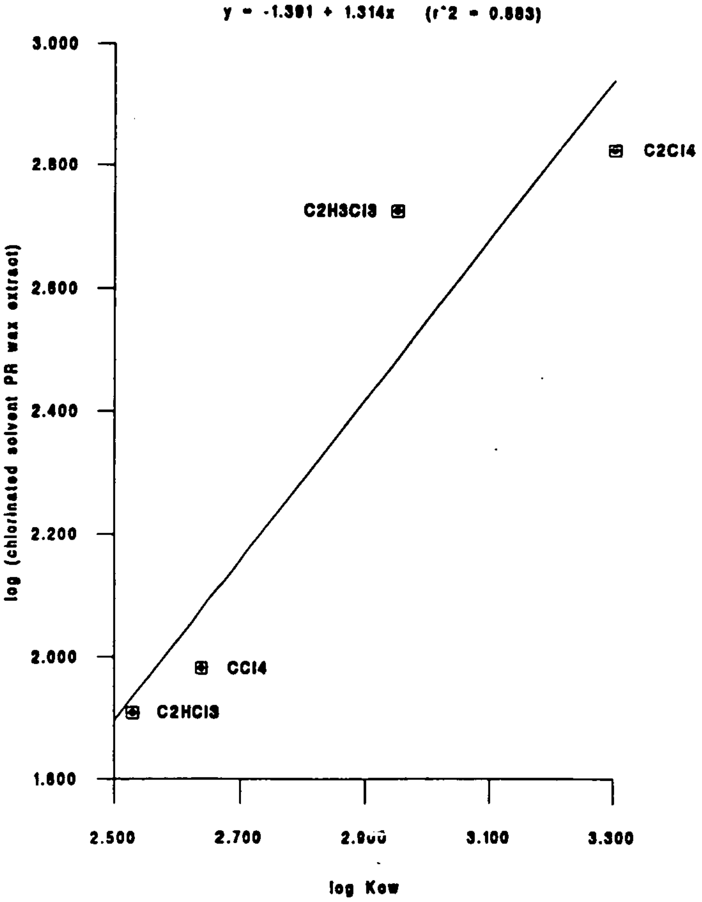


Figure 4.4.1 Log/log correlation between chlorocarbon partition ratio (Scots pine wax extract : air) and K_{ow}.

Better results for the needles are obtained if the partition ratio is assumed to be related to K_{ow} and H (Travis and Hattemer-Frey, 1988): the partition ratio is the ratio of the equilibrium concentration of a chemical in the leaf to its concentration in air. Dividing the two terms by the equilibrium concentration in water, the PR could "also be defined as K_{lw}/K_{aw}, where K_{lw} is the leaf/water partition coefficient, and K_{aw} the air/water partition coefficient. K_{lw} can be quantified by K_{ow}, and K_{aw} is essentially H". Thus (PR.H) should be related to K_{ow}. If H is expressed in the dimensionless (volume/volume) form $K_{aw} = H/RT$, where R is the gas constant 8.314 Pa.m^3/(mol.K) and T is the temperature (K), the PR (gm^{-3}/gm^{-3}), K_{aw} and K_{ow} are all expressed in volume/volume terms and dimensionless.

Following this procedure, an equation was obtained (**Figure 4.4.2**) :

$$\log (\mathrm{PR}.K_{aw}) = -0.701 + 0.853 \log K_{ow} \qquad (r^2 = 0.825)$$

The slope of the correlation above is very near 1, and to a first approximation, the needle/air PR is directly proportional to K_{ow} and inversely proportional to K_{aw} (H), i.e. PR $\cong$ constant.(K_{ow}/K_{aw}). Thus, chemicals with high affinity for lipids (high K_{ow}) need low K_{aw} values to be accumulated to a significant extent and polar organic compounds may have relatively high PRs if K_{aw} is low. The equilibrium leaf/air partition ratio appears to be controlled by two opposing factors : K_{aw} and K_{ow}. When K_{aw} is high, the ecotoxicological potential of highly lipophilic compounds is reduced, and when small, the affinity for lipids, and therefore toxicity, of poor lipophiles is increased.

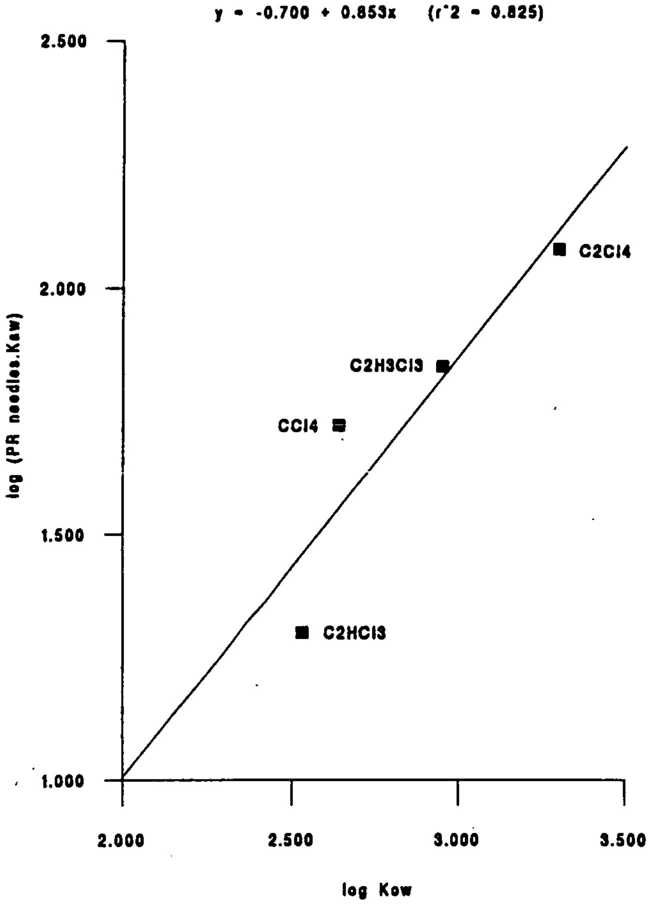


Figure 4.4.2 Relationship between the volume/volume partition ratio of chlorocarbons in Scots pine needles in gm^{-3}/gm^{-3}, the air/water partition coefficient K_{aw} and the octanol/water partition coefficient K_{ow}.

A similar relationship to the one above is not observed for the wax extract, which suggests that K_{ow} as the affinity for lipids is the most important factor controlling equilibrium partitioning into this compartment, rather than K_{aw} (and therefore H). This is interesting considering the wide range of H values investigated (730-13000 Pa.m^3 / mol) and confirms the suggestions in Section 4.3 that the sorption characteristics of the wax extract differ significantly from those of the whole needle.

These results are in agreement with those obtained using higher molecular weight organochlorines distributing into azalea (*Azalea indica*, var. Knut Erwin) (Bacci *et al.*, 1990). It must be remembered however, that the number of chlorinated solvents for which conifer partition ratio data is available is very limited. The chlorocarbon partition ratio results obtained to date by other authors for spruce needles and wax/lipid extract (Figge, 1988; Frank and Frank, 1986) are too sparse to permit any species-specific K_{aw} or K_{ow} correlation comparisons to be made. Further research on the PR characteristics of additional chlorocarbons into Scots pine needles and wax is required before specific partition ratios can be predicted from these correlations.

4.5 Conclusions

(1) The time for an equilibrium partitioning for 4 chlorinated solvents between a Scots pine needle-wax extract and air, and whole Scots pine needles and air was established to be less than 6 hours.

(2) Appropriate partition ratios (volume/volume) were determined using a best fit linear regression. These partition ratios were correlated by log/log plots with K_{ow} , and with H through K_{aw}.

(3) The distribution of chlorocarbons between air and the various compartments of Scots pine needles is, to a first approximation, directly proportional to the octanol/water partition coefficient and inversely proportional to the Henry's law constant (through K_{aw}). That of Scots pine wax is primarily dependent on K_{ow}, reflecting the affinity of chlorinated solvents to lipids. Circumstantial evidence is presented which lends weight to the claims of other authors that several different compartments for sorption within the leaf exist, some of which may be reversible.

Chapter 5

Statistical methods in the analysis of gas chromatography data

All language eventually evolves into algebra.

'Surely,' said the governor, 'Her Radiancy would admit
that ten is nearer to ten than nine is - and also nearer
than eleven is.'
Lewis Carroll

5. STATISTICAL METHODS IN THE ANALYSIS OF GAS CHROMATOGRAPHY DATA

5.1 Introduction

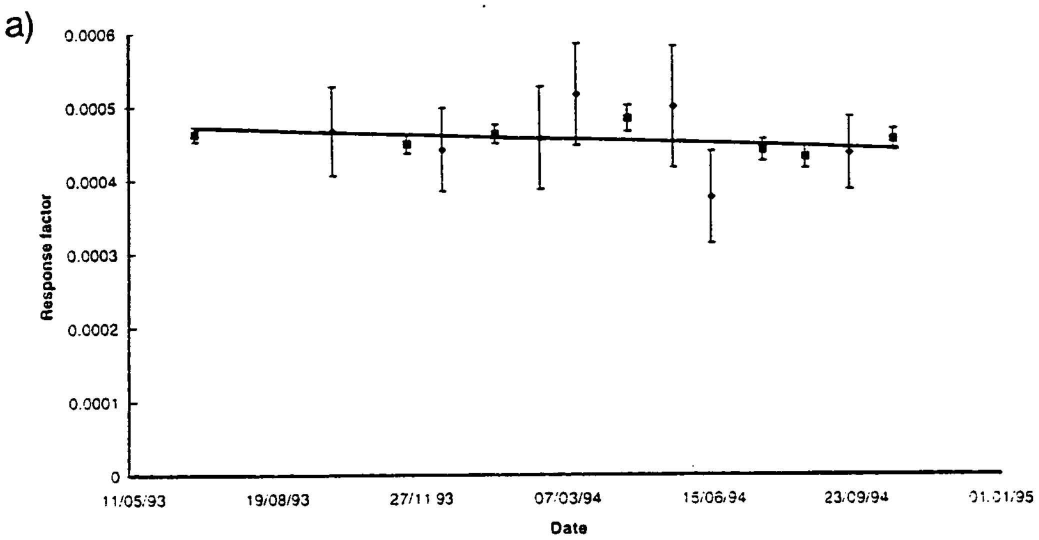
Low limits of detection are a major criterion for successful application of the analytical technique to this environmental problem.

The first section of this chapter describes the use of regression as a parametric method of treating calibration data, and the use of the regression line to calculate the concentrations of chlorinated solvents in needles and air sampler wax by interpolation. The calculation of the limit of detection (LOD) and the analytical limit of detection (ALOD) is considered and an estimate made at the 95% confidence level, using the regression standard error, of the concentration-dependent error associated with the blank-corrected concentrations. The variation of both the CH_3I internal standard and the field blank values over time is presented. A probability plot method used to estimate the geometric mean and standard deviation from censored data is described.

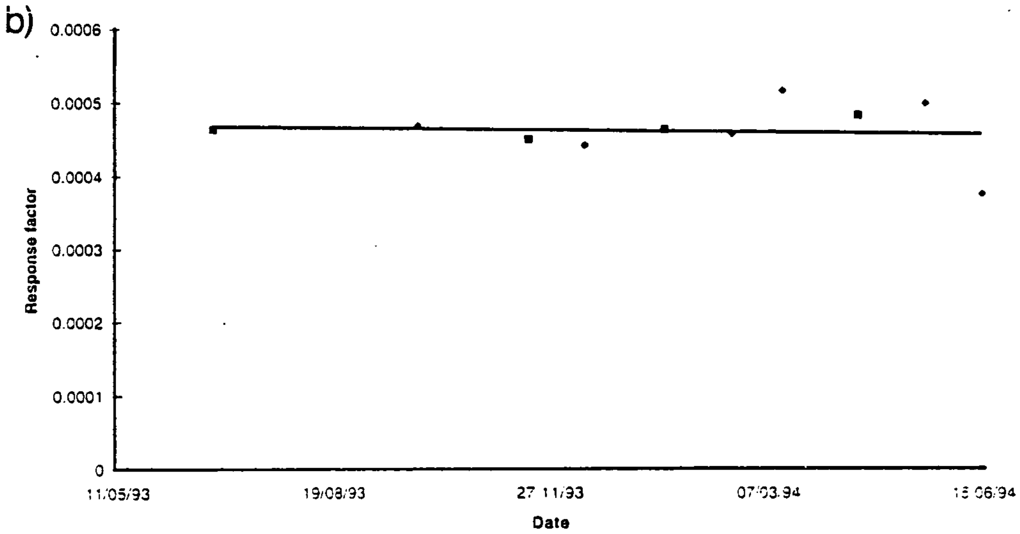
5.2 Calibration data

For each compound, a regression curve of Y (compound peak area/internal standard peak area : internal standard ratio, ISR) on X (compound concentration) was generated for each of the six and three stage calibrations (Chapter 3, Appendix 9). The predicted response factors (slope) were plotted against time and a best fit line constructed using least squares regression to test for systematic changes in response factors with time (**Figure 5.2.1.a**). No significant trend was observed at the 95% confidence level {$p < 0.05$} for any of the compounds studied (only C_2HCl_3 data are shown in **Figure 5.2.1**), although in some cases a slight slope was observed (negative or positive). The trends either side of the change of analytical column on 19/07/94 were also checked (**Figure 5.2.1(b,c)**) and no significant differences were seen. Similarly, it was established that the predicted intercept showed no significant trend over time (**Figure 5.2.2(a-c)**). This indicates that the analytical procedure was consistent during the year and that there was no major systematic error. The standard error of each of the six and three stage calibration regression curves, as plotted in the Figures, provides an estimate of the random error. The accuracy is seen to be greater on the 6 stage calibrations (error is about ± 10%) where as the error on the 3 stage calibrations is ± 30%.

Based on this information, the calibration data for each compound over the whole analytical period were combined to provide a better time-averaged estimate of the response factor and intercept for concentration calculations and to improve the overall accuracy (**Figure 5.2.3a**). **Figure 5.2.3b** shows an example of a six stage calibration plot.



Variation of response factor for C2H3Cl3 between 25/06/93 and 14/06/94



Variation of response factor for C2H3Cl3 from 20/07/94 to 19/10/94

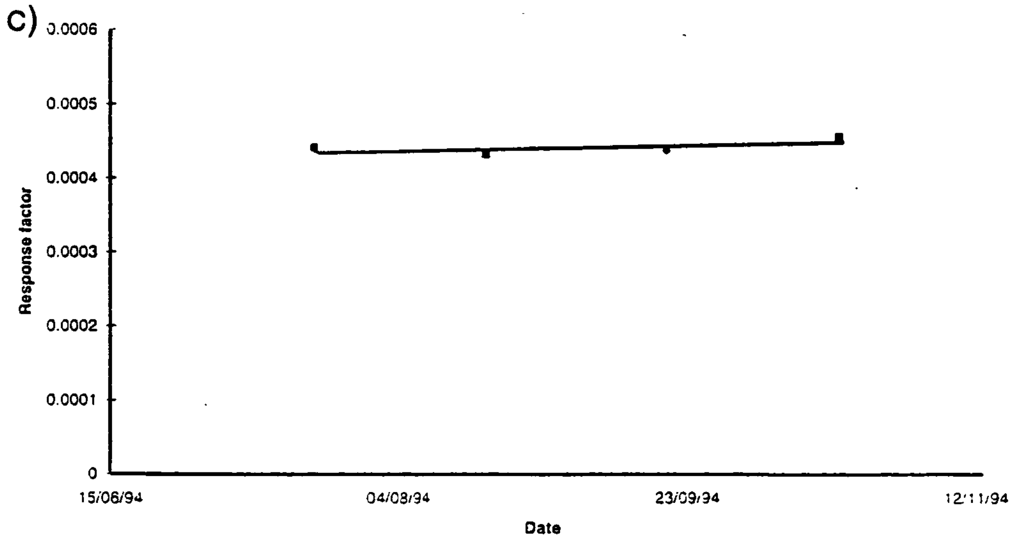
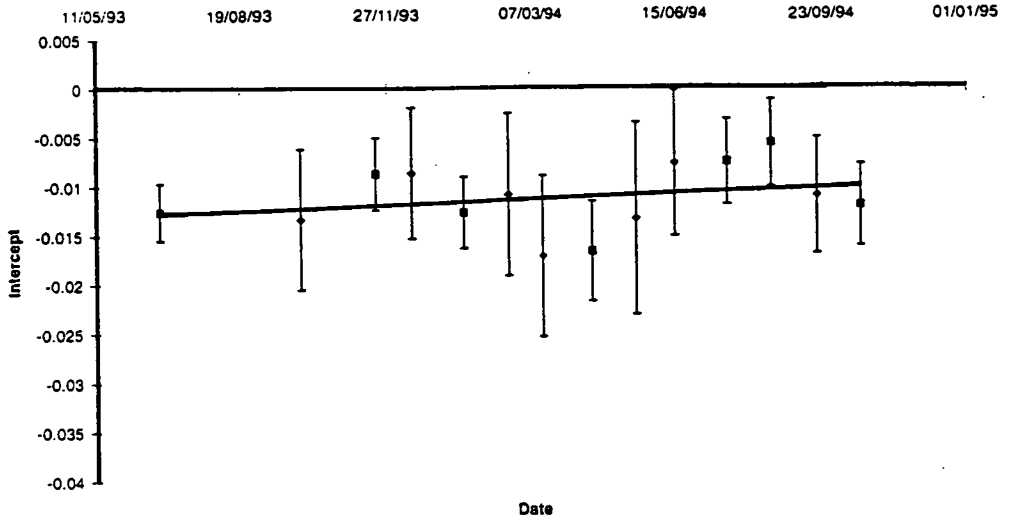
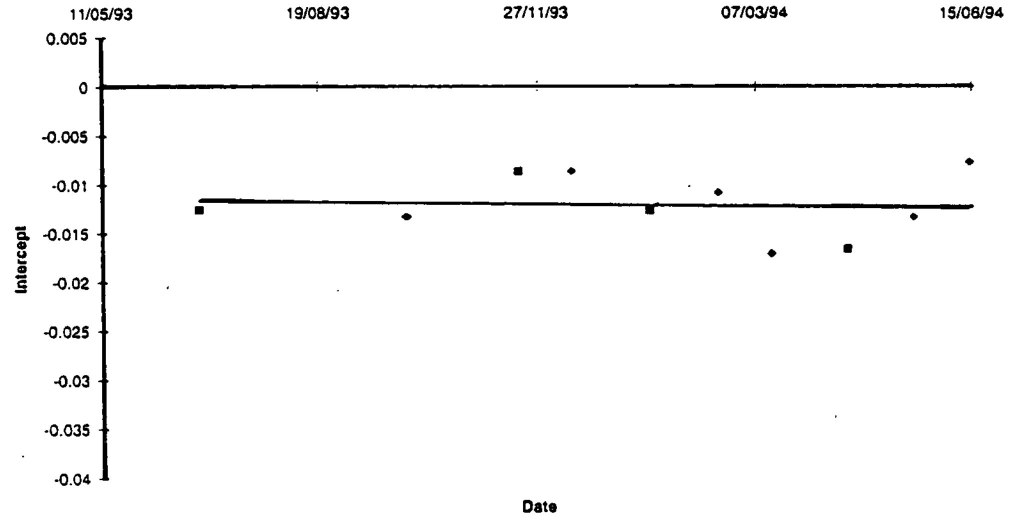


Figure 5.2.1a Predicted response factor trend over time using $C_2H_3Cl_3$ as an example. The error bars show the standard error of response factor from each calibration. Note that the standard errors are considerably greater on the 3 point (◆) rather than the 6 point (■) calibrations. Graphs **b** and **c** show trends before and after the column change. None of the trends are significant at the 95% confidence level.

Variation of intercept with time for C2H3Cl3



Variation of intercept for C2H3Cl3 between 25/06/93 and 14/06/94



Variation of intercept for C2H3Cl3 between 14/06/94 and 19/10/94

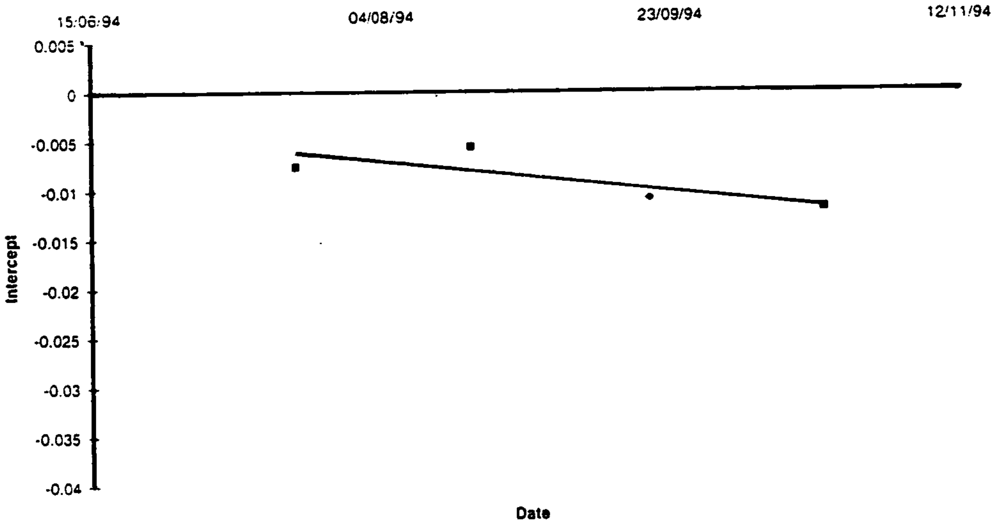
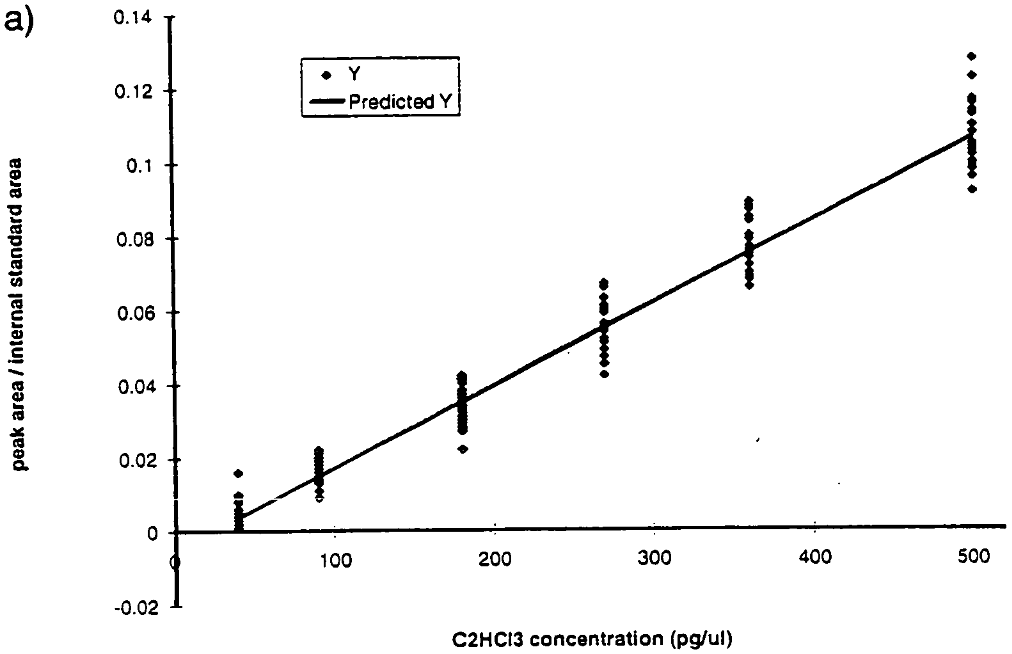


Figure 5.2.2 Predicted intercept trend over time using $C_2H_3Cl_3$ as an example. Again the 6 point (■) calibrations show greater accuracy (smaller intercept standard errors for each calibration) than the 3 point (◆) calibrations. None of the trends are significant at the 95% level.

a)



b)

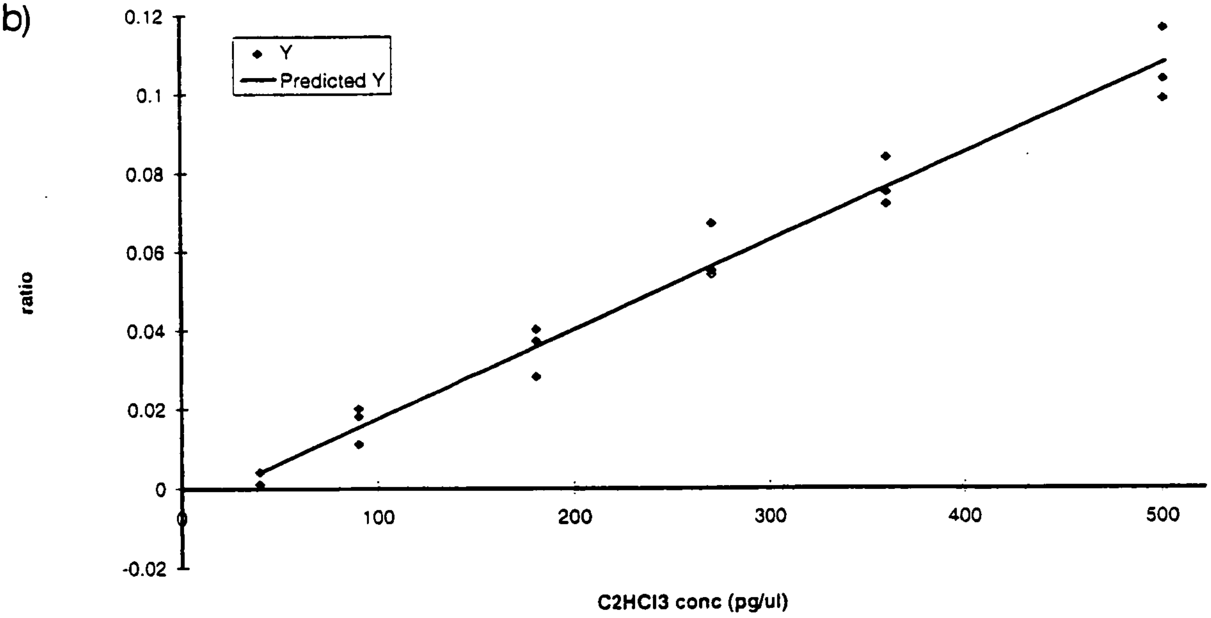


Figure 5.2.3a,b Example of a full calibration curve (for C_2HCl_3) generated using regression over all six and three stage calibrations, and a typical six stage curve, showing 3 replicate injections of 6 different analytical standards on 25/06/93.

The mean response factor and intercept calculated for each compound over all time, with the standard error on each as a measure of the uncertainty, are summarised in **Table 5.2.1**. These data are used to estimate the concentrations of the test samples. The values for the intercepts are all non-zero and significant at the 95% confidence level, and the regression line is not forced through zero. As all sample analyses are compared against blanks, the absolute value of the intercept is not included in final reported values (sample - blank).

Table 5.2.1 Response factor and intercept with standard error for each compound, calculated using regression over all time.

	Response factor (x1000)	**Standard error on response factor (x1000)**	**Intercept (x1000)**	**Standard error on intercept (x1000)**
$CHCl_3$	0.48	0.0057	21	1.5
$C_2H_3Cl_3$	0.46	0.0046	-11	1.2
CCl_4	2.1	0.015	-45	3.9
C_2HCl_3	0.23	0.003	-5.3	0.76
C_2Cl_4	0.57	0.0075	22	1.9

The small values of the standard errors for each compound (typically 2% for the response factor and <10% for the intercept) show that the data are tightly grouped about the mean.

As a further check for systematic differences over time, the independent single point values of the daily (180 pg/injection) calibration check analyses for each compound were plotted against time, and compared with the predicted ratio response calculated for a concentration of 180 pg/injection from the combined regression using 3 point and 6 point calibrations. The 95% confidence limits were calculated using the error on a single prediction and applying this to the predicted response ratio. The error on a single prediction is needed for the 180 pg/µl point, as the standard error of the regression is based on a pivotal mid-point:

Error on a single prediction = $t_{0.05,n} \times S$

Where S = $S_{yx}\sqrt{1 + 1/n + x^2 / \sum x^2}$

n = points on calibration curve (168)

S_{yx} = standard error (from regression)

x = $180 - \bar{x}$ (180 is concentration at which response is predicted)

$\bar{x}$ = mean concentration of calibration points

$\sum x^2$ = $\sum (x - \bar{x})^2$ for all 168 points on line (x = concentration)

Example calculation for C_2Cl_4 (Figure 5.2.4)

Error = $1.97 \times S$ = 0.0292

Where S = $0.148\sqrt{\left[1 + 1/168 + \left((180 - 206)^2 / 3970052\right)\right]}$

Thus the 95% limits are given by the predicted ratio response (0.125) ± 0.0292.

The check values for all the compounds were shown to be distributed within these limits over all time.

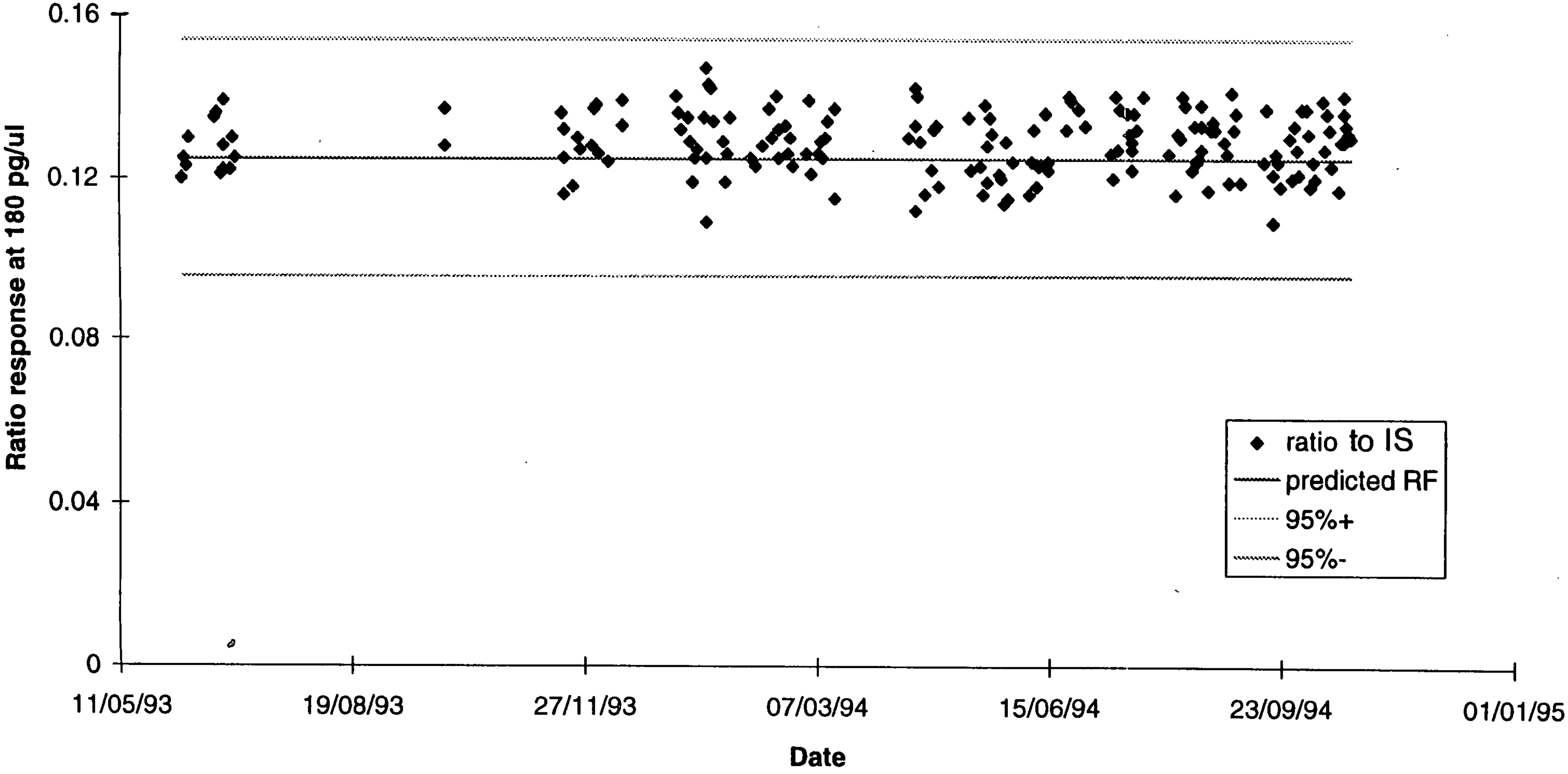


Figure 5.2.4 Variation of independent check values for C_2Cl_4 about the predicted ratio response. The 180 pg/µl data is taken from the full calibration data set in Appendix 9.

5.3 Analytical limit of detection (ALOD)

The analytical limit of detection is defined as the absolute ability of the data handling capacity of the GC integrator to detect a peak based on the set parameters for peak recognition/rejection. Any values that are reported as zero by the peak integrating system are in fact 'less-thans'. The peak area reject parameter of the integrating system was set to zero during the entire analytical period, and the area sensitivity was 693 counts in the test area for positive peak identification. The area (and baseline) sensitivity is calculated automatically at minimum background noise and drift. The mean area for the internal standard is 3.7×10^7 (±50%) counts (194 pg/injection), and the corresponding ISR values for each compound can be used, with the appropriate response factor, to set the lower analytical limit of detection (ALOD) for each compound, based on the lower limit ratio for peak detection of $693/3.7 \times 10^7 = 1.9 \times 10^{-5}$ (**Table 5.3.1**). These values are at least an order of magnitude lower than the absolute blank detection limits (see Chapter 2).

Table 5.3.1 Analytical limit of detection values for needle and wax samples. Conversions to concentrations in ng/g were based on approximately 1g fresh weight of needles per vial in 2ml desorption solvent, and 0.12g wax per air sampler in 1ml desorption solvent.

	Compound peak area/ internal standard peak area ratio (x1000)	**Response factor** (x1000)	**pg/ injection**	**Needle concentration** ng/g fresh weight	**Air sampler wax concentration** ng/g wax
$CHCl_3$	0.019	0.48	0.040	0.08	0.3
$C_2H_3Cl_3$	0.019	0.46	0.041	0.08	0.3
CCl_4	0.019	2.1	0.009	0.02	0.08
C_2HCl_3	0.019	0.23	0.083	0.2	0.7
C_2Cl_4	0.019	0.57	0.033	0.07	0.3

The ALOD (and absolute blank detection limit) for CCl_4 is up to an order of magnitude less than that of the other four compounds. This reflects the enhanced response of the ECD to this compound (Table 3.1.1 Appendix 4), also seen in the high sensitivity (slope of calibration regression curve, **Table 5.3.1**) relative to the other chlorinated solvents.

The standard deviation of the mean internal standard value over all time is approximately 50% (**Figure 5.3.1**) which must be applied to the estimated ALOD. The wide variation in the internal standard area indicates that the absolute response of the detector varies on a day-to-day basis. During any given analytical day, the internal standard area remains reasonably constant (±10%). The observed variation between days and over all time is too great to be accounted for solely by injection technique, and justifies the use of the internal standard method. The full data set is given in Appendix 9.

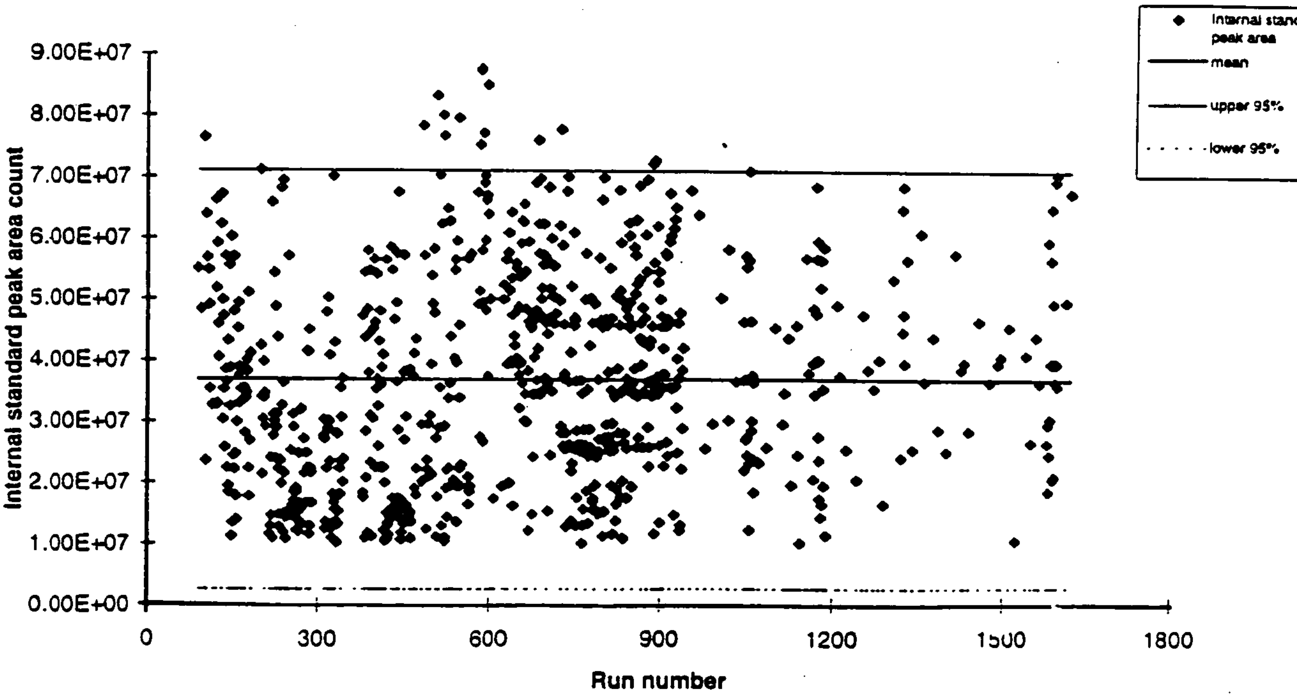


Figure 5.3.1 Variation of internal standard peak area over all time.

5.4 Field blanks and limit of detection (LOD)

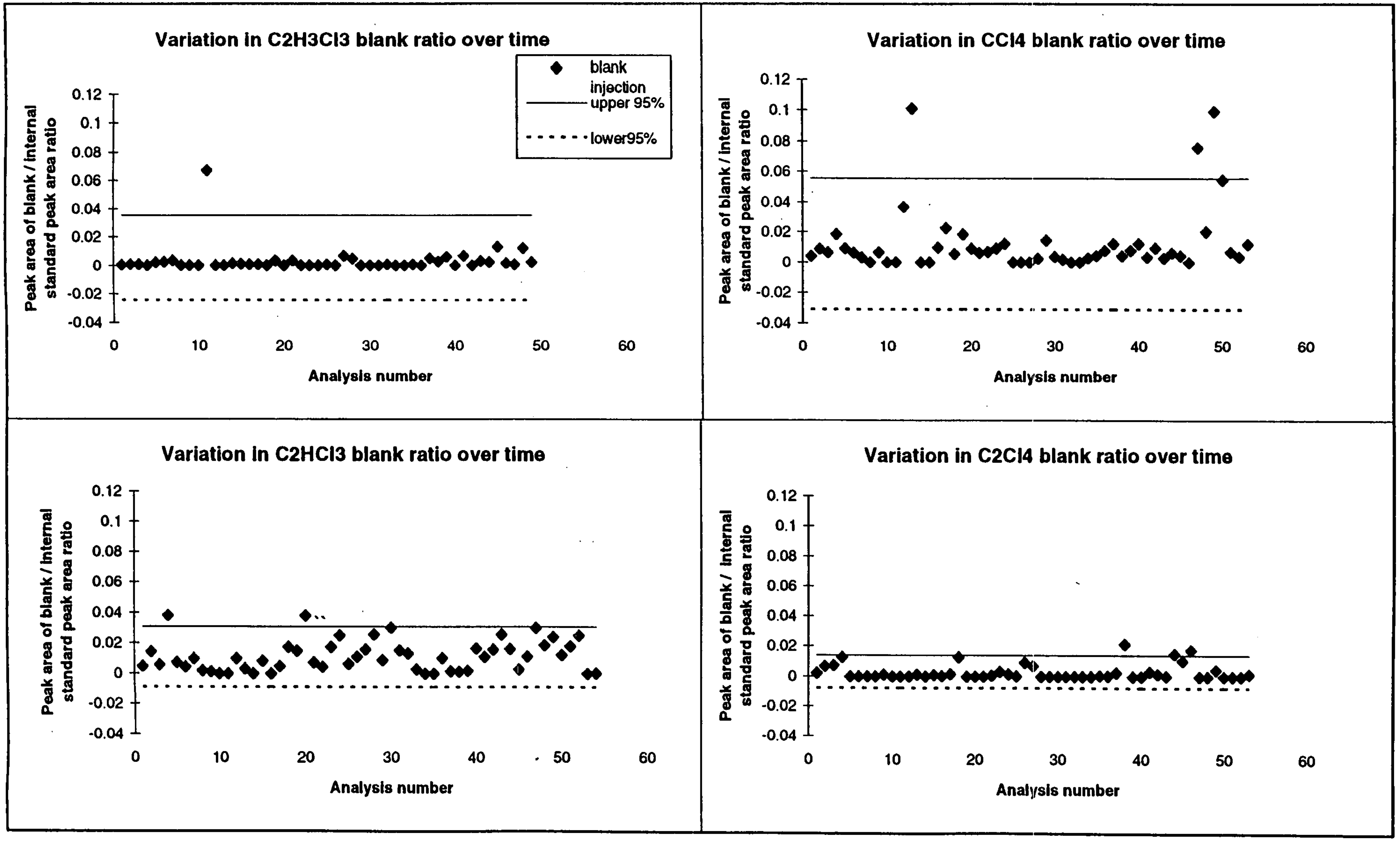
When using a regression line for calibration, a fundamental assumption is that each point on the plot, including that representing the background (blank), has a normally distributed variation in the y direction at fixed x with a standard deviation $S_{y/x}$ (Miller and Miller, 1994):

$$S^2_{y/x} = \left[\frac{\sum_i (y_i - \hat{y}_i)^2}{n - 2} \right]$$

This equation uses the y residuals ($y_i - \hat{y}_i$) where the $\hat{y}_i$ values are the points on the calculated regression line corresponding to the individual fixed *x*-values, i.e. the fitted *y*-values.

A commonly used definition in analytical literature for the limit of detection (LOD) of a method is 'the analyte concentration giving a signal equal to the blank signal, y_B, plus two standard deviations of the blank, s_B' (Miller and Miller, 1994). It is possible to use $S_{y/x}$ in place of s_B in the estimate of the LOD and the intercept as a more accurate estimate of y_B (Miller and Miller, 1994). However, in this study, y_B and s_B as the mean and standard deviation (coefficient of variation) were retained in the LOD calculation for each sampling occasion, being more representative of the large field blank day-to-day variation arising from handling of samples in the field. The reason for using the field blanks is that it is assumed that these are contaminated in the field to the same extent as the sample.

The variation of the field blank ratios over all time shows that values generally fall within the 95% bounds (**Figure 5.4.1**). A series of t-tests (two-tailed, unpaired) performed on the blank data confirmed that overall no significant differences {$P<0.05$} existed between sampling occasions at any site (Appendix 10). The mean blank value over all time was not adopted for the same reasons as y_B and s_B were retained for the LOD calculation. The value adopted for the blank mean must be flexible to reflect the day-to-day variability and give a true measure of the real concentrations observed in the field.


Variation in C2H3Cl3 blank ratio over time

Variation in CCl4 blank ratio over time

Variation in C2HCl3 blank ratio over time

Variation in C2Cl4 blank ratio over time

Figure 5.4.1 Variation of field blank ratios over time for each compound.

5.5 Example of needle and sampler data

The following procedure is shown as an example ($C_2H_3Cl_3$ concentration at Banchory in 1992 needles, March sampling 1993) in Appendix 11. For each sampling occasion, the three peak ISR values obtained from the individual injections of needle blanks (field blanks) or air sampler blanks (sampler blanks) were each converted to pg/injection using the slope and intercept of the compound regression curves :

$$X = \frac{Y - b}{c}$$

where X = concentration (pg/injection)
Y = ISR
b = intercept
c = fitted slope (response factor)

Similarly, the needle and sampler wax peak area ISR values from each injection were converted into pg/injection. The values for y_B (mean of the blank) and s_B (standard deviation of the blank) were calculated from the pg/injection data. The value of y_B was used to correct the needle and sampler data; each individual needle or wax result was corrected before calculation of the mean and standard deviation for the sampling occasion:

Field (or sampler) blank-corrected value = needle (or wax) value - y_B
(pg/injection) (pg/injection)

These field (or sampler) blank-corrected values were converted to ng/g dry weight (needles) or ng/g wax (sampler) as described in Chapter 3 (see Appendix 2 and 6). The blank-corrected ng/g values were used for all the statistical tests.

As stated, the LOD (in pg/injection) for each compound at each sampling occasion is calculated as (y_B+2s_B) allowing it to reflect the actual blank values on that day. If the concentration (ISR) for the injection of a field blank is less than that for the lab blank (i.e. there is contamination in the standards used for lab calibration) then the effective field blank concentration is negative, and field sample concentrations are increased accordingly. The variation of the (negative) field blank is used to define the LOD in exactly the same way as when the field blank concentration is greater than the lab blank concentration. If the individual component ratios in the sample are less than those of the field/sample blank then the corrected concentrations are negative values. When the field blanks are high it may mean that the observed sample value is higher than it actually appears.

As the associated error on these pg/injection values is concentration-dependent, the estimate of the 95% confidence limits on the corrected concentrations generated refers to the standard error (a point estimate) from the relevant component calibration curve. This provides an estimation of the error arising on an individual value from both the slope and intercept and therefore the precision of an individual concentration.

Using the regression equation of Y (ratio) on X (concentration), the estimated concentration from a given ISR is designated $\hat{X}$. The 95% confidence level for $\hat{x}$, where $\hat{x} = \hat{X} - \overline{X}$ and $\overline{X}$ is the mean of all X is given as follows (Miller and Miller, 1994):

$$x = \frac{\hat{x} \pm \left(t.S_{yx} / b\right) \left[\left\{ (n+1)/n \right\}(1 - c^2) + \hat{x}^2 / \sum x^2 \right]^{1/2}}{1 - c^2}$$

Where t = Student's t statistic at the 95% level for (n-2) degrees of freedom

n = number of points on calibration curve

$$c^2 = \left[\frac{1}{\sum x^2}\right]\left[\frac{t.S_{yx}}{b}\right]^2$$

b = fitted slope

$\sum x^2$ = [sum of squared X-values; $\sum X^2$] - [(sum of X values)^2/n; $\frac{(\sum X)^2}{n}$]

$\sum y^2$ = [sum of squared Y-values; $\sum Y^2$] - [(sum of Y values)^2/n; $\frac{(\sum Y)^2}{n}$]

$$\sum xy = (\sum X - \sum Y) - \frac{(\sum X . \sum Y)}{n}$$

$S^2_{y/x}$ = $\frac{\sum dyx^2}{n-2}$ [(standard error of regression curve)^2]

where $\sum dyx^2 = \sum y^2 - \frac{(\sum xy)^2}{\sum x^2}$

5.6 Problems with $CHCl_3$ and contamination

Representative concentrations of $CHCl_3$ cannot be determined by the methods described (Chapters 2 and 3) due to the contamination levels of the blank (**Figure 5.6.1**). The variation of peak area/internal standard ratio (ISR) over time shows a repeating pattern of reasonable constancy followed by a rapid increase. When a new internal standard stock solution is made up (e.g. at runs 325, 430, 590 and 900, **Figure 5.6.1**) the ISR value drops significantly to initial levels. This suggests that there is gradual contamination of the internal standard stock solution with concomitant increase in field blank levels. The source of the contamination is probably laboratory air, as the adjacent chemistry laboratory regularly used large amounts of this compound as a solvent, which could enter the internal standard vial in increasing amounts as the septum on the vial became progressively more fractured. No similar effects for the other 4 compounds was seen.

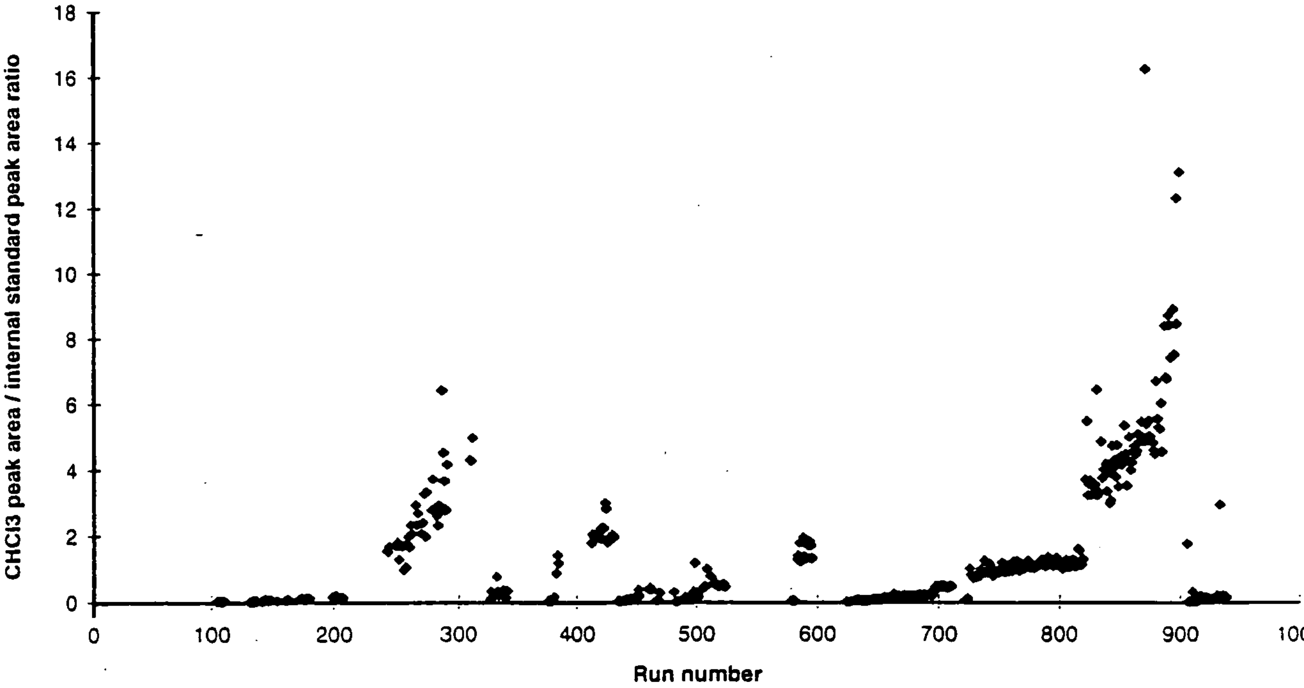


Figure 5.6.1 The $CHCl_3$ peak area/internal standard peak area ratio (ISR) for all needle samples and calibration samples plotted across time.

The needle concentration data set does fall approximately within two standard deviations of the mean of the blank, technically allowing a < value to be established, or even an estimated absolute number by difference (i.e. subtraction of a constant, or increasing at a constant rate, blank value). However, as the values obtained would only be approximations at best, and erroneous at worst, the $CHCl_3$ data was discarded at this stage.

As **Table 5.2.1** shows, the $CHCl_3$ calibration data is a tight fit about the mean as the standards were made up containing the appropriate amount of internal standard, i.e. not made up separately as a stock solution and drawn up as with the field samples.

5.7 Probability plot method for estimating geometric mean and standard deviation from censored data

Having obtained the concentrations of the different analytes in all the needle samples and wax tube samples, it was observed that many results were below the defined limit of detection (LOD). In comparing data among sites it was necessary to calculate an unbiased estimate of the distribution means and standard deviations (Porter *et al.*, 1988).

Data that is technically below the LOD can be incorporated for the purposes of estimation, hypothesis testing and regression (Helsel, 1990). When measurements below the LOD are reported as not detected, or less-thans, then the information contained in that data is lost. It is better to report numbers, even if some of those numbers are very imprecise (Porter *et al.*, 1988). It is important that an estimate of precision is given for every measurement; in the results presented below, the 95% confidence level is calculated for each sampling occasion.

As is characteristic for trace gases in air (Fowler and Cape, 1982; Helsel, 1990), the data set for each compound shows an approximately log normal distribution (see example in **Figure 5.7.1**), and an adaptation of a probability plot method is used to fit a distribution to the data above the LOD.

In the probability plot method, the fitted line is used to extrapolate a collection of below-limit values assuming a distributional shape. These values ('fill-ins') are not considered estimates for specific samples but are combined with the above LOD data only to estimate summary statistics. The robustness of this method results from use of all observed data rather than a fitted distribution above the LOD. This makes it less sensitive to the fit of a distribution for the largest observations. The mean and standard deviation (moment statistics) are very sensitive to values of the largest observations, so failure of the assumed distribution to fit these observations will result in poor estimates. In addition, it also avoids transformation bias, e.g. between measurement scales, by performing all computations of summary statistics in the original units (Helsel, 1990).

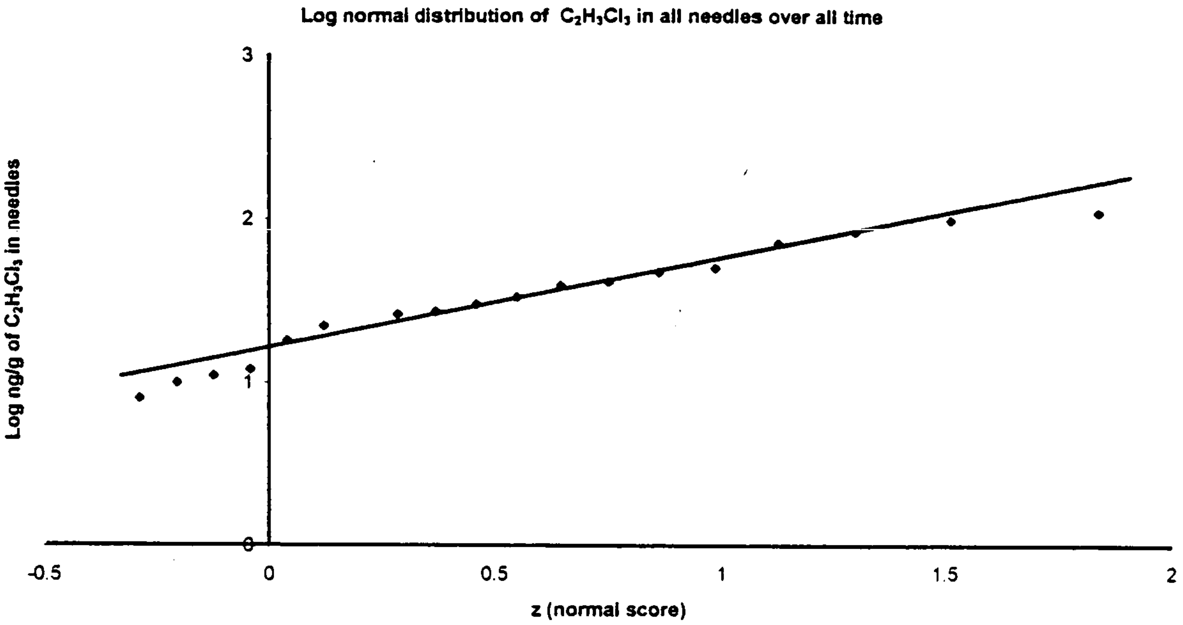


Figure 5.7.1. Log normal distribution of the data set for $C_2H_3Cl_3$.

The robust probability plot method has been recommended for estimating the geometric mean and standard deviation (Travis and Land, 1990) and is the procedure adopted in this study.

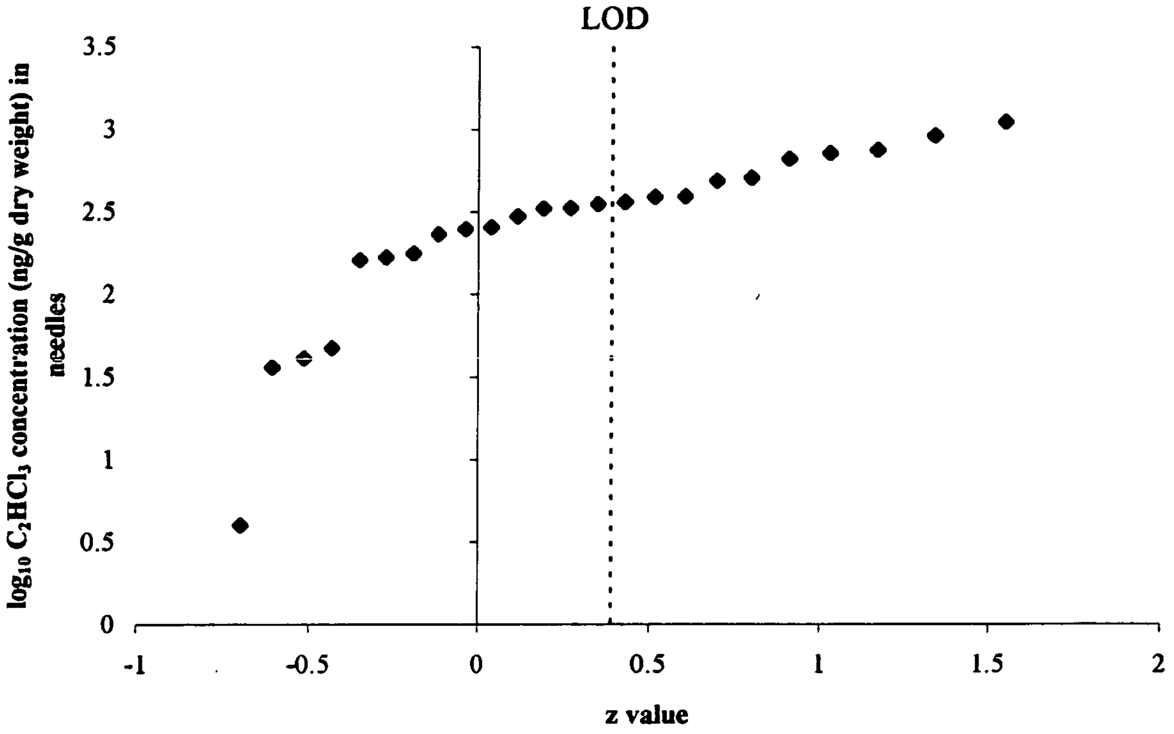
Normal (z) scores are calculated for all the observed blank-corrected data (ng/g) in the set including values below the LOD (see example below) (Porter *et al.*, 1988). A linear regression is performed for log concentrations greater than the LOD against the standard normal variate (z). The mean and standard deviation of the log values are given by the intercept and slope respectively. As the regression allows the *y* axis (log concentration) to intersect the *x* axis (normal score) at zero, the 50% value (log geometric mean) is given by the intercept, and the geometric standard deviation by the slope. By definition, the median has 50% of the values of the data above it and 50% below. The geometric mean (and standard deviation) can be converted to the median values in original units (ng/g) without transformational bias. They are expressed as logarithmic ($\log_e$) values for the purposes of hypothesis testing (Chapters 6 and 7).

In many cases, values below the defined LOD appear to be part of the same distribution, i.e. fall close to the line defined by the points above the LOD. These values were included in the regression estimate to increase the number of degrees of freedom. In the example below, the upper 10 points used in generating the regression line are >LOD, and the remaining 5 that are included fall in the 'fill-in' (<LOD) part of the probability plot. The severe tailing of the probability plot is typical and such data are obviously excluded from the regression calculation.

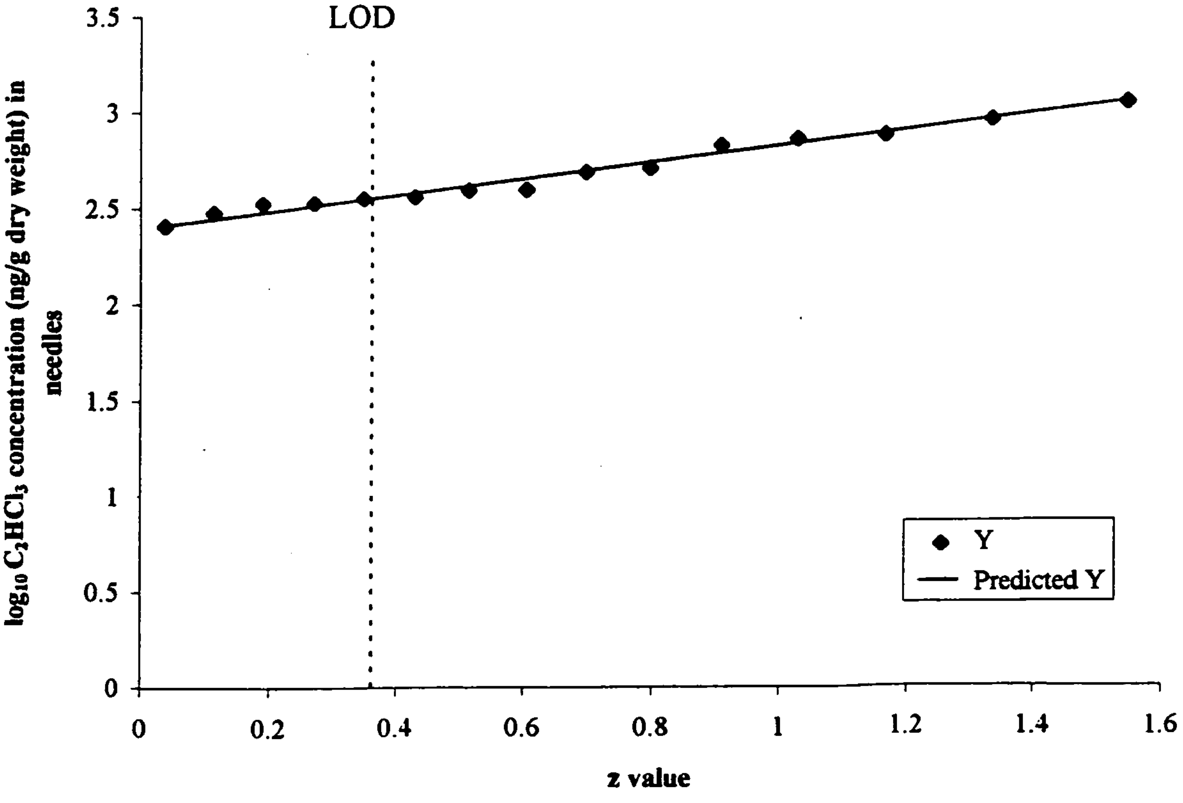
Example of C2HCl3 calculated population over all time at Ponsonby Tam forest								
1992	conc							
needles	(ng/g)	*Point*	*Column1*	*Rank*	*Percent*	z	log10 conc	
	5000	1	5000	1	100.00%			
01/04/93	232	21	1129	2	96.90%	1.546432	3.052694	
	335	6	920	3	93.90%	1.334624	2.963788	
	394	19	760	4	90.90%	1.165047	2.880814	
	333	14	724	5	87.80%	1.027893	2.859739	
	920	25	669	6	84.80%	0.907769	2.825426	
05/06/93	167	18	511	7	81.80%	0.796056	2.708421	
	41	23	486	8	78.70%	0.696684	2.686636	
23/09/93	354	4	394	9	75.70%	0.603765	2.595496	
	-141	15	391	10	72.70%	0.51293	2.592177	
	297	16	362	11	69.60%	0.428895	2.558709	
	248	9	354	12	66.60%	0.347787	2.549003	
	177	3	335	13	63.60%	0.268908	2.525045	
	724	5	333	14	60.60%	0.189118	2.522444	
	391	11	297	15	57.50%	0.113039	2.472756	
	362	22	255	16	54.50%	0.037608	2.40654	
16/02/94	47	12	248	17	51.50%	-0.04012	2.394452	
	511	2	232	18	48.40%	-0.11556	2.365488	
	760	13	177	19	45.40%	-0.19167	2.247973	
	-308	7	167	20	42.40%	-0.27151	2.222716	
	1129	24	161	21	39.30%	-0.35045	2.206826	
	255	17	47	22	36.30%	-0.43164	1.672098	
	486	8	41	23	33.30%	-0.51579	1.612784	
	161	30	36	24	30.30%	-0.60678	1.556303	
	669	27	4	25	27.20%	-0.69988	0.60206	
23/05/94	-24	29	-7	26	24.20%	-1.55477		
	4	26	-24	27	6.00%	-1.55477		
	-24	28	-24	27	6.00%	-1.55477		
	-7	31	-24	27	6.00%	-1.55477		
	36	32	-24	27	6.00%	-1.55477		
	-24	33	-24	27	6.00%	-1.55477		
	-24	34	-24	27	6.00%	-1.88079		
	-24	10	-141	33	3.00%			
	-24	20	-308	34	.00%			
Regression Statistics								
Multiple R	0.989872					mean of log10 values		2.401572
R Square	0.979846					sd of log10 values		0.418592
Adjusted R	0.978296							
Standard E	0.028593					median conc		252.0995
Observatio	15					geom sd		2.621755
ANOVA						loge geom mean		5.529824
	df	*SS*	*MS*	*F*	*ignificance*	loge geom sd		0.963844
Regressior	1	0.516731	0.516731	632.0374	2.08E-12			
Residual	13	0.010628	0.000818			95% confidence		1.927688
Total	14	0.52736						
	Coefficients	*tandard Err*	*t Stat*	*P-value*	*Lower 95%*			
Intercept	2.401572	0.013309	180.4417	1.75E-23	2.372819			
X Variable	0.418592	0.01665	25.14035	2.08E-12	0.382622			
Upper 95%	*wer 95.00*	*per 95.000%*						
2.430325	2.372819	2.430325						
0.454563	0.382622	0.454563						

Example of C2HCl3 calculated population over all time at Ponsonby Tarn forest (continued)

Probability plot for all C_2HCl_3 data in Ponsonby Tarn 1992 year class needles across all time



Regression plot using censored data to calculate $\log_e$ geometric mean from the intercept, and $\log_e$ standard deviation from the slope



For each compound (excluding $CHCl_3$), the median needle concentration through the year each of the sites was calculated and plotted as the natural log showing the 95% confidence intervals (twice the geometric standard deviation). Log geometric mean needle concentrations were also calculated for each compound at each site for all sampling occasions where there was sufficient data for regression. These log-transformed geometric means formed the basis of the t-tests used for comparison of populations at the 95% confidence level.

The air sampler tubes were analysed as two distinct data sets :

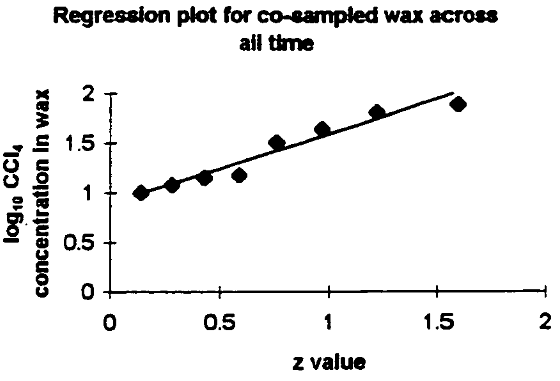
(1) tubes sampled at the same time as foliage;

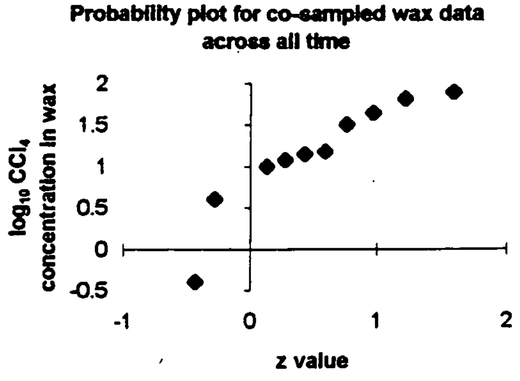
(2) tubes sampled at other times.

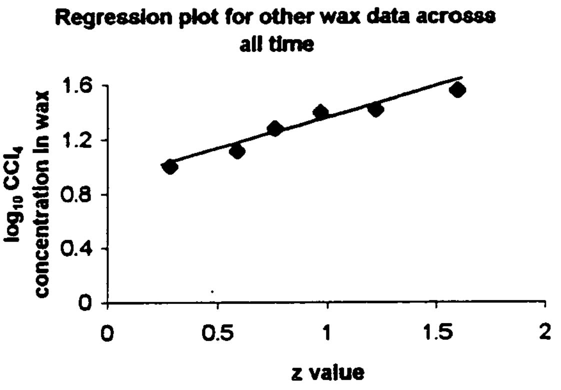
The geometric mean and standard deviation for each data set were determined separately. If the two data sets were not significantly different (population t-test) a combined geometric mean and standard deviation were calculated (see example below).

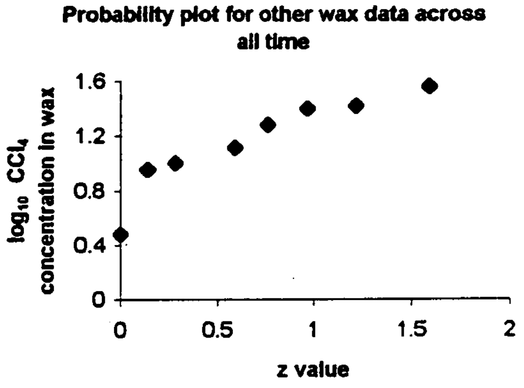
The probability plot method is used to calculate the ln geometric mean of CCl4 concentration in wax for the co-sampled and other data sets.

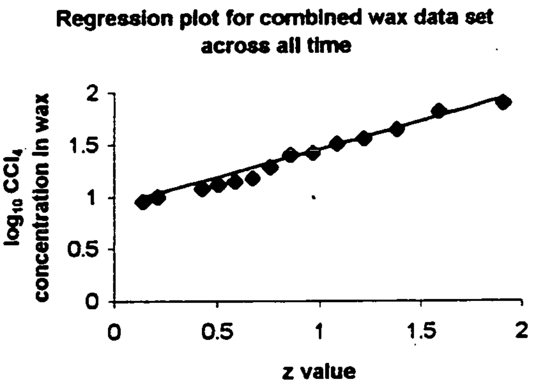
The population t-test finds no significant difference exists between the data sets, and the two data sets are combined. The ln geometric mean of this combined data set is then calculated.

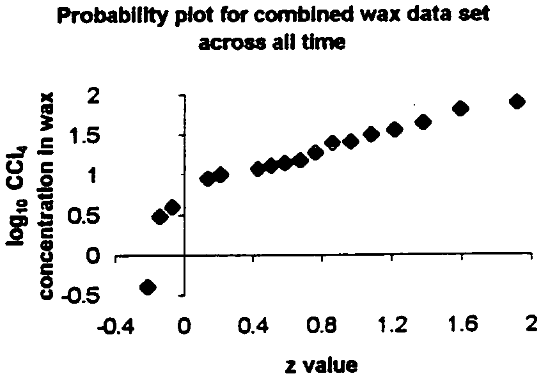












Co-sampled data		Other data		Combined data	
mean of log values	0.88945	mean of log values	0.892	mean of log values	0.8613
sd of log values	0.69321	sd of log values	0.442	sd of log values	0.5646
median conc	7.75266	median conc	7.798301	median conc	7.266077
geom sd	4.93415	geom sd	2.766942	geom sd	3.669442
ln geom mean	2.04804	ln geom mean	2.053906	ln geom mean	1.983217
ln geom sd	1.59618	ln geom sd	1.017743	ln geom sd	1.30004
95% confidence	3.19236	95% confidence	2.035485	95% confidence	2.600079

Chapter 6

Results and discussion : Chlorinated solvents in pine needles

I went to the woods because I wished to...see if I could
not learn what it had to teach.
Henry David Thoreau

6. RESULTS AND DISCUSSION : CHLORINATED SOLVENTS IN PINE NEEDLES

The results obtained for chlorinated solvent concentrations in whole needles are presented, interpreted and discussed.

6.1 Results

The field blank-corrected results in ng/$g_{dry\ weight}$ for $C_2H_3Cl_3$, CCl_4, C_2HCl_3 and C_2Cl_4 at each of the three forests are given in **Tables 6.1.1-3**. The mean and standard deviation of the triplicate analyses are shown in bold, values >LOD are in italics. The < values are the ALOD for that compound as determined by GC integration parameters (Table 5.3.1). The full data set before blank-correction is tabulated in Appendix 9. **Table 6.1.4** summarises the $\log_e$ geometric mean concentrations (ng/g) estimated using the probability plot method over the total population at each forest. This data for the two needle classes sampled is presented graphically in **Figure 6.1.1**. The 95% confidence limits (twice the geometric standard deviation) are shown in the Figure.

TABLE 6.1.1

Field blank-corrected data in ng/g dry weight

< values are analytical limit of detection (ALOD) i.e. determined by GC integration parameters

values >LOD in italics

Mean and standard deviation of replicate injections in bold

BANCHORY

	$C_2H_3Cl_3$		CCl_4		C_2HCl_3		C_2Cl_4	
	1992	1993	1992	1993	1992	1993	1992	1993
25/03/93	*30*		3		*327*		*52*	
	13		-0.2		73		23	
mean	**22**		0.1		*176*		**38**	
sd	**12**		**1**		**192**		**20**	
			2		**128**			
31/05/93	*35*				132		38	
	31	-10	-11		173		48	
	33		-13		97		-23	
	3		**-11**		**134**		**21**	
			1		**38**		**38**	
04/11/93	124	-244	3	-33	-35	32	5	*72*
	93	-163	-31	36	*127*	76	*94*	*99*
	-257	-257	1	35	91	73	*137*	*125*
	-13	**-221**	**-9**	**12**	**61**	**60**	**79**	**98**
	211	**51**	**19**	**40**	**85**	**25**	**67**	**27**
24/01/94	*20*	8	-3	-3	124	-43	*16*	2
	53	-4	7	-3	*278*	63	-2	3
	28	*29*	**2**	-3	87	43	**7**	**2**
	33	**11**	**7**	**-3**	**163**	**21**	**13**	**1**
	17	**17**		**0**	**101**	**56**		
09/05/94	*13*	<0.08	7	7	-51	-51	*15*	*32*
	35	*6*	12	-4	-46	-51	<0.07	<0.07
	<0.08	*18*	8	6	-17	-4	*114*	**16**
	16	**8**	9	**3**	**-38**	**-35**	<0.07	**23**
	18	**9**	**9**	**6**	**19**	**27**	**32**	
			2				**55**	

TABLE 6.1.2

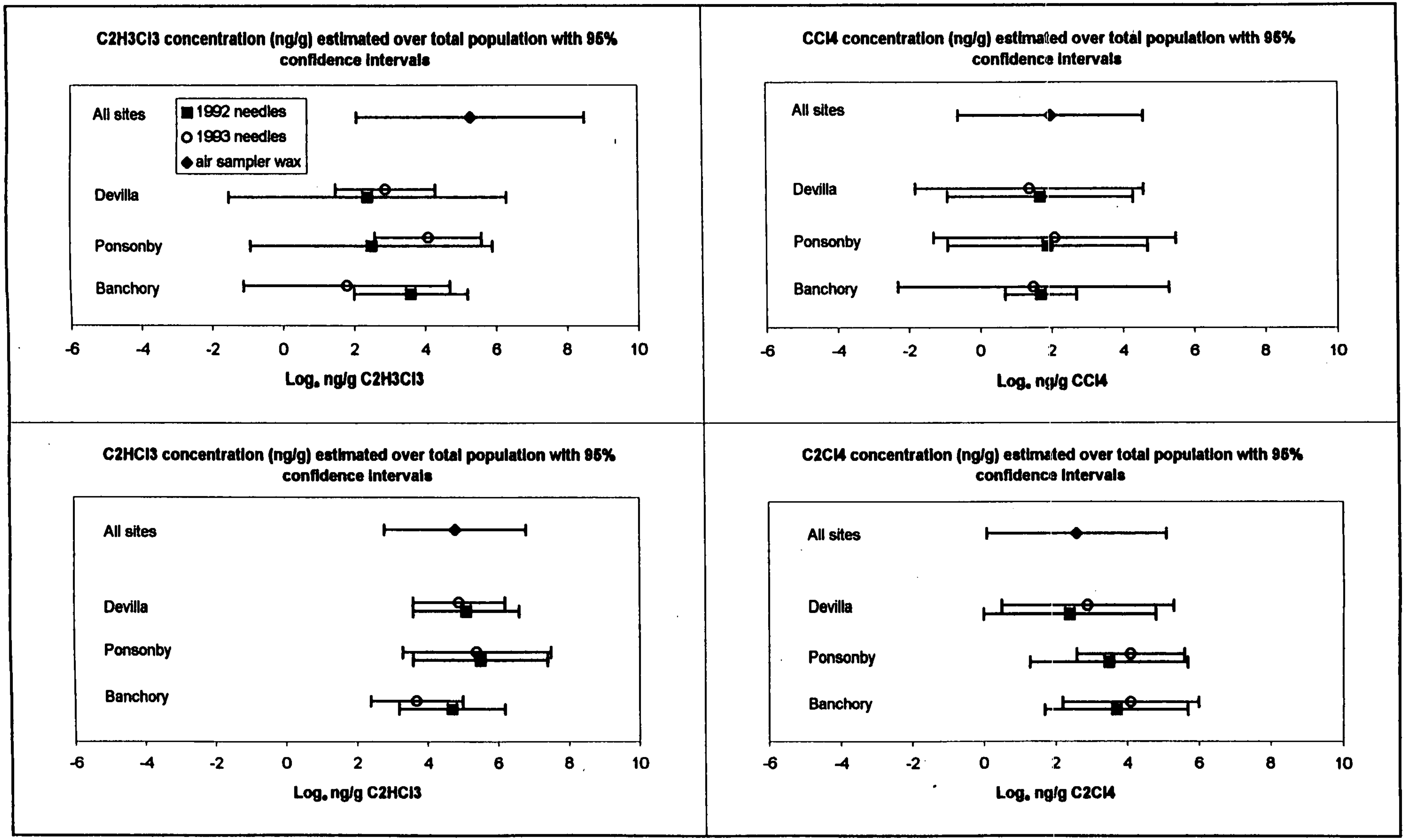
PONSONBY TARN FOREST																								
			C2H3Cl3						CCl4						C2HCl3						C2Cl4			
	west		central		east		west		central		east		west		central		east		west		central		east	
	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993
01/04/93	27		5				-21		-23				232		333				-9		48			
	-4		-6				-19		-21				335		920				13		7			
	55		-0.4				-16		-14				394		626				2		28			
mean	26		7				-19		-19				320		416				16		29			
sd	29						3		6				82											
05/06/93	-28						-26						167						30					
	-28						-21						41						-3					
	-28						-14						104						18					
	-28						-20						89						29					
	0						6																	
23/09/93	-1		18		-1		<0.02		<0.02		<0.02		354		248		391		6		-15		11	
	-1		-1		-1		<0.02		77		<0.02		-141		177		362		-15		95		19	
	-1		311		56		<0.02		38		<0.02		297		724		376		-6		170		17	
	-1		109		18		0		64		0		170		383		21		16		83		16	
	0		176		33								271		297						93		4	
16/02/94	48	25	-14	64	71	29	34	2	-6	18	10	27	47	417	-308	513	486	-261	-19	92	37	72	58	14
	15	-4	88	126	21	48	17	5	53	69	16	35	511	20	1129	40	161	742	135	7	125	143	51	2
	16	64	114	117	26	38	16	8	23	119	17	31	760	447	255	1516	669	548	7	50	82	-16	64	8
	27	28	63	103	39	13	22	6	42	68	14	6	439	296	359	690	439	343	41	60	81	66	6	8
	19	34	68	33	27		10	3		61	3		362	238	724	764	267	632	83		44	80		
23/05/94	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08	-1	12	3	6	11	7	-24	-24	-7	-19	-24	-24	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
	11	<0.08	<0.08	<0.08	<0.08	<0.08	3	9	13	13	8	11	4	-21	36	18	-24	-14	<0.07	<0.07	14	<0.07	<0.07	34
	<0.08	<0.08	14	47	<0.08	<0.08	15	-3	8	9	7	-3	-24	-5	-24	-24	-24	10	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
	4	<0.08	5	16	<0.08	<0.08	6	6	8	9	9	5	-14	-17	2	-8	-24	-8	<0.07	<0.07	6	<0.07	<0.07	11
	6	0	8	27	0	0	8	8	5	4	2	7	16	10	31	23	0	18	0	0	8	0	0	20

TABLE 6.1.3

DEVILLA FOREST		C2H3Cl3				CCl4				C2HCl3				C2Cl4		
	1992 13m	1993 13m	1992 10m	1993 10m	1992 13m	1993 13m	1992 10m	1993 10m	1992 13m	1993 13m	1992 10m	1993 10m	1992 13m	1993 13m	1992 10m	1993 10m
15/03/93	21		-3		0.3		11		347		221		25		5	
	104		219		5		-1		529		397		9		20	
	170		-3		13		16		438		364		32		12	
mean	98		71		6		9		128		327		22		10	
sd	75		128		7		8				94		12			
11/06/93	-42		-30		-5		11		15		200		20		-52	
	-20		35		3		-4		355		57		12		-62	
	-31		-33		-9		3		543		592		16		-57	
	16		-9		-4		4		304		283		6		7	
			38		6		8		268		277					
11/08/93	-1	-1	49	-1	<0.02	<0.02	<0.02	<0.02	110	-37	30	25	-1	9	-4	39
	182	-1	71	56	<0.02	<0.02	<0.02	<0.02	-22	139	-180	-32	7	17	-9	-0.1
	-1	-1	72	42	<0.02	<0.02	<0.02	<0.02	94	307	29	80	3	113	-6	40
	60	-1	64	63	<0.02	<0.02	0	0	60	137	-40	24	6	46	4	26
	106	0	13	40	0	0			72	172	121	56		58		23
				28												
23/11/93	-12	-3	251	-10	59	59	-72	9	141	283	325	60	56	89	16	17
	-7	-12	-15	-13	42	1	29	-35	201	239	107	-133	71	69	-46	-9
	5	-7	118	-8	37	19	-95	38	422	335	216	78	113	94	-15	50
	-5	6	188	-10	46	26	-46	4	255	286	154	2	80	84	43	19
	9			3	12	29	66	37	148	48		117	29	13		30
10/02/94	-13	26	13	-32	4	19	12	0.02	162	-35	99	165	5	47	11	-5
	16	-15	3	-44	5	2	5	1	278	197	-49	42	4	7	12	15
	301	-33	20	-38	5	10	14	-1	240	499	261	63	23	10	13	3
	101	-7	12	9	1	12	10	0.01	227	220	104	90	11	21	12	4
	173	30	9				5	0.6	59	268	155	65	11	22	1	10
21/04/94	-8	45	-6	-6	5	1	-3	2	91	231	-18	19	40	16	<0.07	<0.07
	30	16	-6	-6	3	-0.07	2	1	184	100	-257	110	37	18	<0.07	15
	-8	-8	36	8	2	4	4	3	-50	60	3	311	10	52	12	<0.07
	5	18	8	-1	3	2	1	2	75	131	-90	146	29	29	4	5
	21	27	24	8	1	2	4	1	118	89	144	149	17	20	7	9
31/05/94	-2	0.2	14	-2	15	7	4	4	-2	-0.2	-2	-1	-3	-3	21	6
	-2	35	-2	-2	16	9	3	-1	-0.4	-2	-2	-1	-3	-3	-3	-3
	-2	32	-2	5	11	1	1	0.2	-2	-0.3	-1	-2	16	31	3	20
	-2	-2	3	0.4	14	25	3	1	-2	-1	-1	-1	3	8	5	8
	0	2	9	4	3	11	1	2	1	1	0.3	0.4	11	8	14	12
		14				10								16		

Table 6.1.4 Log$_e$ and back-transformed geometric mean and standard deviation of chlorinated solvent concentrations in pine needles (ng/g dry weight).

Compound	Forest	Year class			
		1992		1993	
		Log$_e$ geometric mean and sd concentration	Geometric mean and sd	Log$_e$ geometric mean and sd concentration	Geometric mean and sd
$C_2H_3Cl_3$	**Banchory**	3.7±0.7	35 ^{x}/2.2	1.8±1.4	6 ^{x}/4.2
	Ponsonby	2.5±1.8	12 ^{x}/5.6	4.2±0.7	57 ^{x}/2.1
	Devilla	2.5±1.8	12 ^{x}/6.9	3.0±0.7	18 ^{x}/2.0
CCl_4	**Banchory**	1.6±0.5	5 ^{x}/1.6	1.6±1.8	5 ^{x}/6.8
	Ponsonby	1.8±1.4	6 ^{x}/4.0	2.1±1.6	8 ^{x}/5.5
	Devilla	1.6±1.4	5 ^{x}/3.7	1.4±1.6	4 ^{x}/5.0
C_2HCl_3	**Banchory**	4.6±0.7	106 ^{x}/2.1	3.7±0.7	40 ^{x}/1.9
	Ponsonby	5.3±0.9	245 ^{x}/2.2	5.5±1.2	231 ^{x}/2.9
	Devilla	5.1±0.7	161 ^{x}/2.1	4.9±0.7	137 ^{x}/2.0
C_2Cl_4	**Banchory**	3.7±0.9	39 ^{x}/2.7	4.2±0.9	58 ^{x}/2.6
	Ponsonby	3.5±1.2	31 ^{x}/3.0	4.2±0.7	59 ^{x}/2.1
	Devilla	2.3±1.2	11 ^{x}/3.3	2.8±1.2	18 ^{x}/3.2



C2H3Cl3 concentration (ng/g) estimated over total population with 95% confidence intervals

CCl4 concentration (ng/g) estimated over total population with 95% confidence intervals

C2HCl3 concentration (ng/g) estimated over total population with 95% confidence intervals

C2Cl4 concentration (ng/g) estimated over total population with 95% confidence intervals

Figure 6.1.1 Log_e geometric mean concentrations in pine needles through the year. Log_e geometric mean concentrations in sampler wax (Chapter 7) are shown for comparison.

Selected examples showing the typical trends observed between sites, heights and year class at the three forests are graphically presented in **Figures 6.1.2-7**. The first Figure (**Figure 6.1.2a**) shows an example of a typical pg/injection against time plot. Similar plots were generated for each compound by forest, needles class, site and height as appropriate.

In this example, the CCl_4 concentration in Ponsonby needles at the 10m sampling height is plotted for each site (west, central and east) for the two dates on which 1993 year class needles were collected. Each point on the graph represents an individual injection. Three injections of the same volume of solvent used to extract the needle sample were made; these are shown in a vertical group adjacent to the corresponding blank group.

The corresponding $ng/g_{dry\ weight}$ plot is shown in **Figure 6.1.2b**. Note that approximately half the values are below the LOD. These values are included in the graph to help visual identification of trends. Typically 30-50% of the plotted values are above the LOD. Also note that, for this particular example (**Figure 6.1.2a**), two analyses (A and B) of needles sampled on 23/05/94 contained less CCl_4 than the mean of the field blank analyses. This is reflected in **Figure 6.1.2b** where two negative ng/g values (A' and B') are seen. The spread of results from three injections of the same volume of the same extractant can be marked. This is seen particularly well for the central site sampled on 16/02/94.

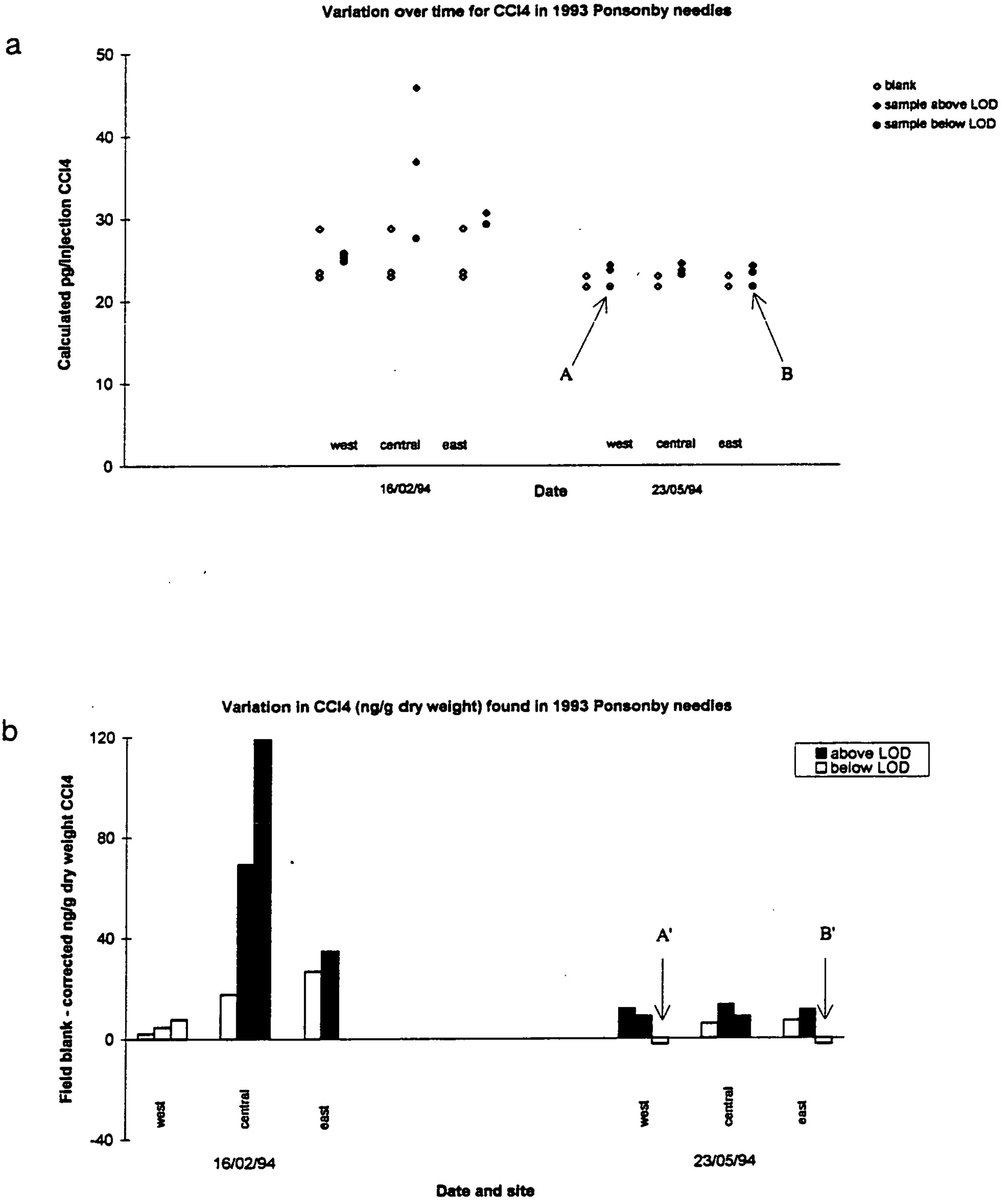


Figure 6.1.2(a,b) The pg/injection and corresponding ng/g plot across time for CCl_4 concentrations in 1993 year class needles at Ponsonby Tarn forest.

For comparison, the ng/g plot for the CCl_4 concentrations found in 1992 year class needles from Ponsonby forest is in **Figure 6.1.3**.

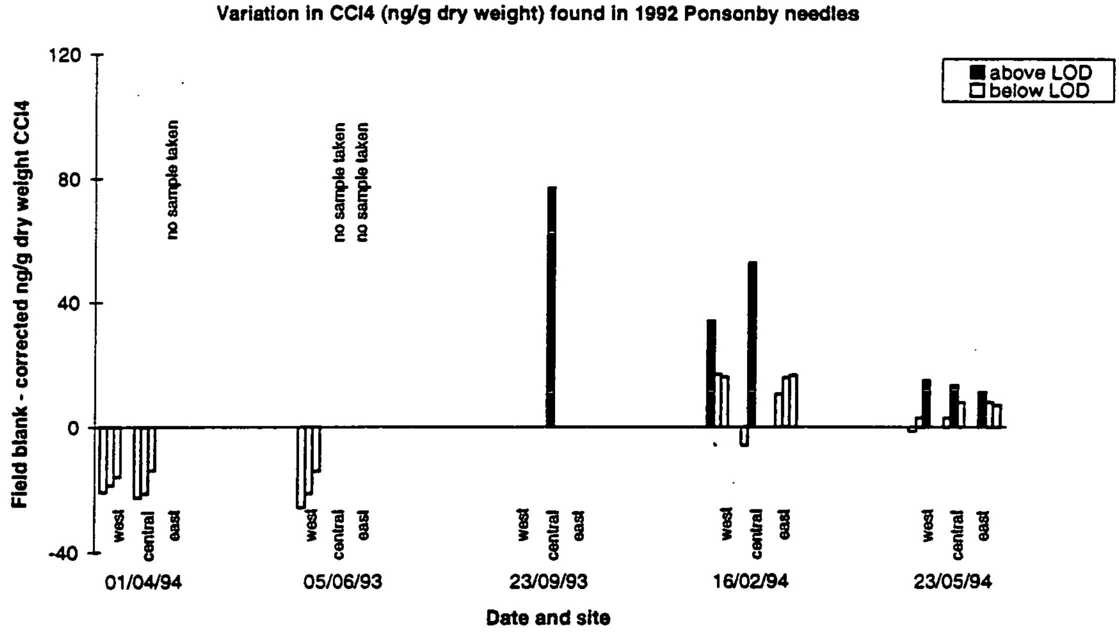


Figure 6.1.3 The ng/g plot across time for CCl_4 concentrations in 1992 year class needles at Ponsonby Tarn forest.

As a further example from Ponsonby forest, ng/g plots for C_2HCl_3 in 1992 and 1993 needles are shown in **Figure 6.1.4**.

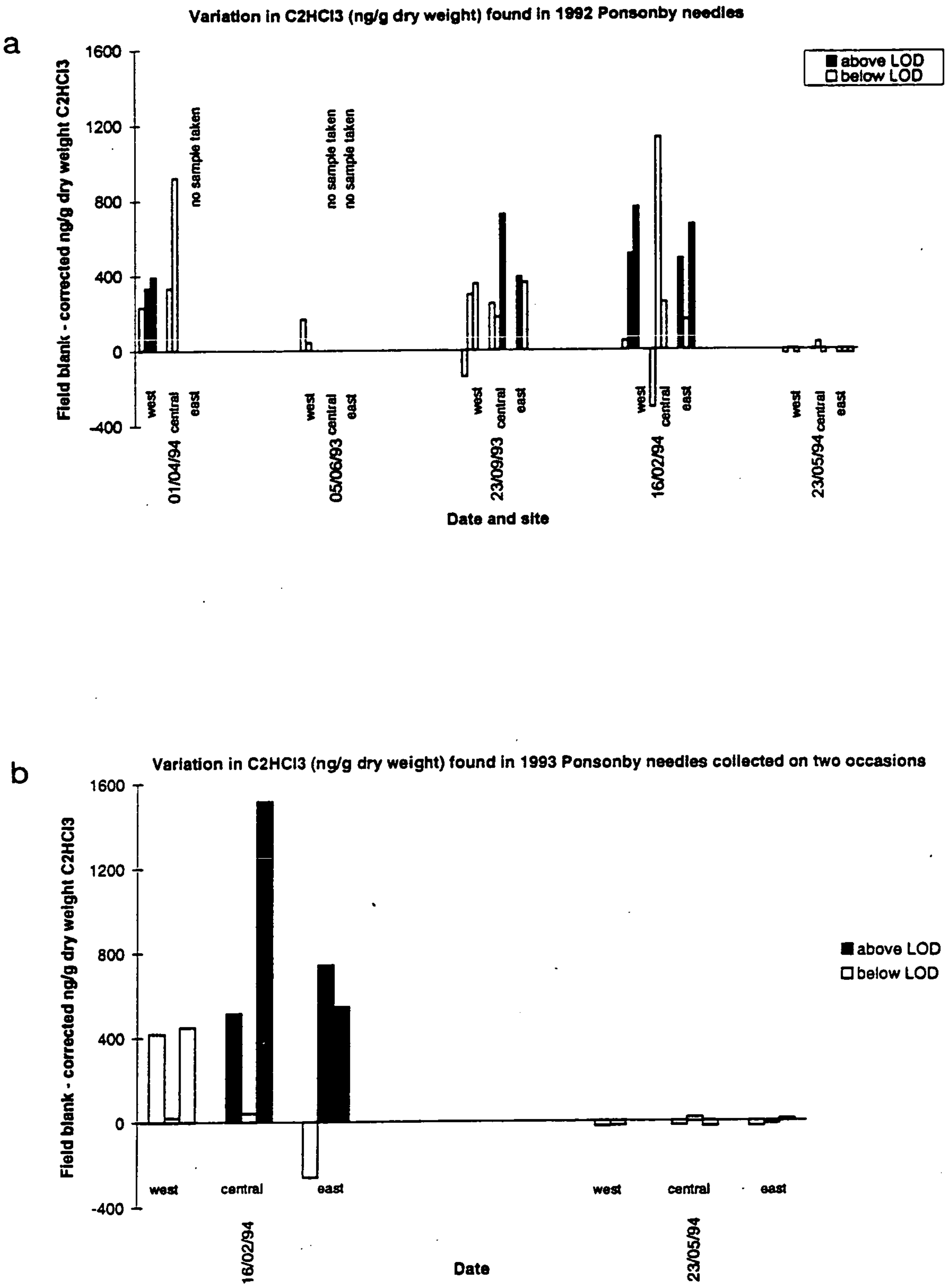


Figure 6.1.4(a,b) ng/g plot for C_2HCl_3 in 1992 and 1993 year class needles collected in Ponsonby Tarn forest.

Examples from Banchory forest for $C_2H_3Cl_3$ and CCl_4 concentrations in both needle classes are shown in **Figure 6.1.5(a,b)**. Note the difference in scale of the concentration (*y*) axis between the two plots.

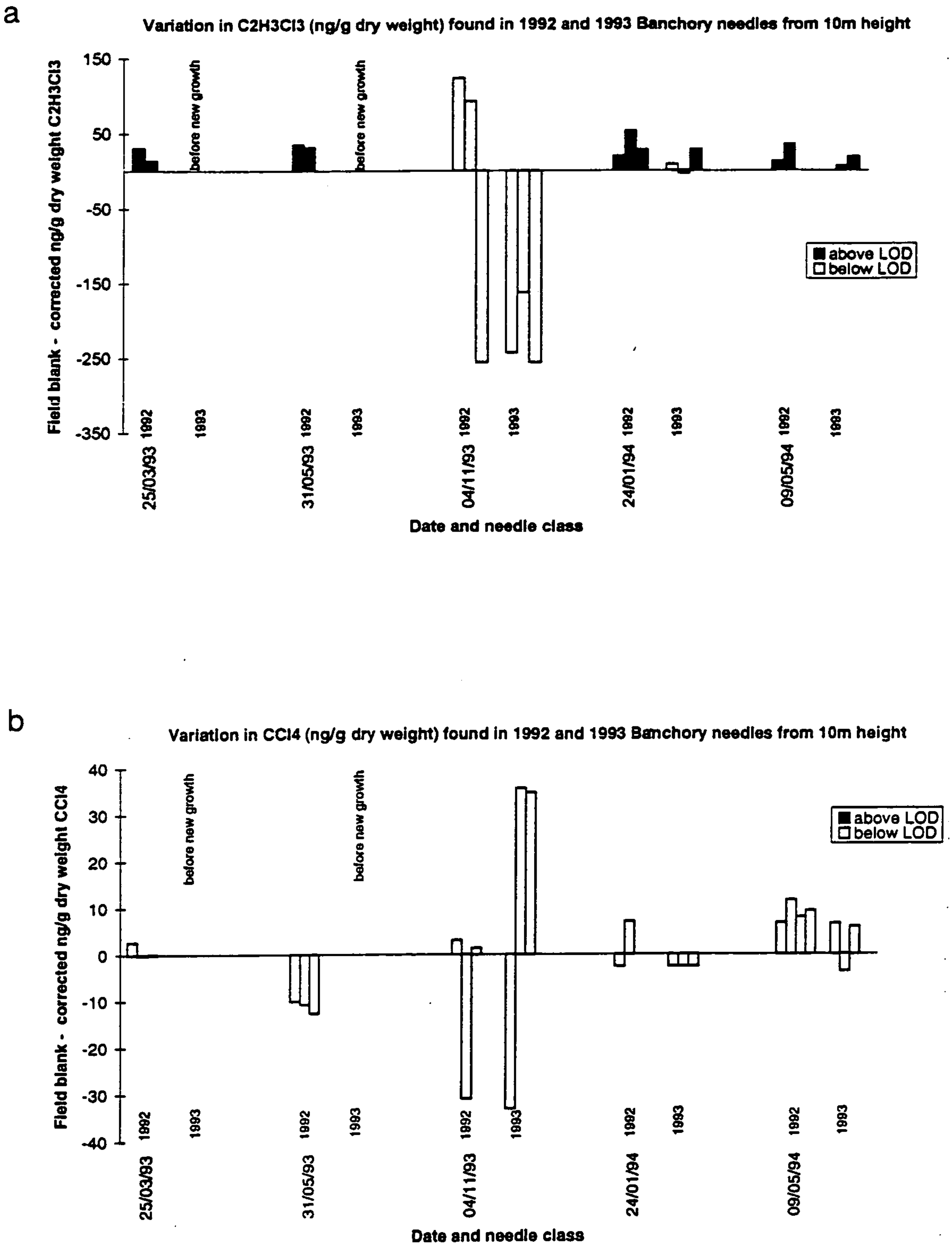
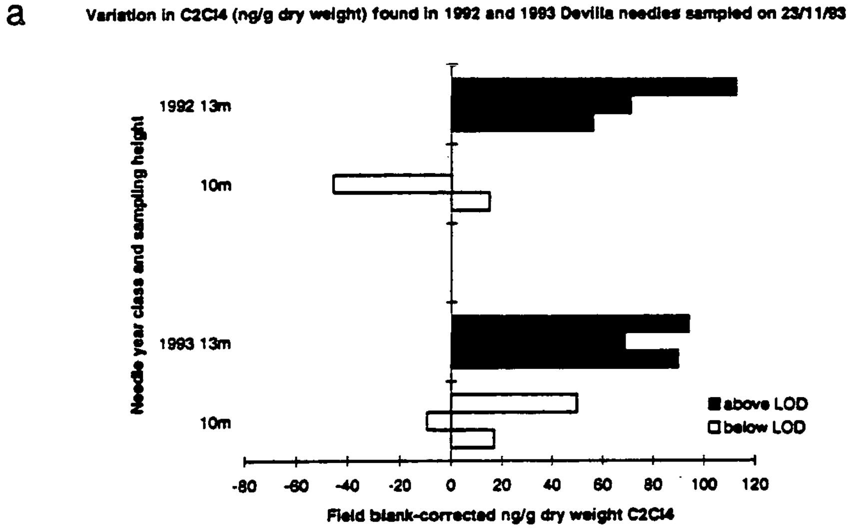


Figure 6.1.5 Variation in (a)$C_2H_3Cl_3$ and (b)CCl_4 concentrations found in 1992 and 1993 year class needles from Banchory forest.

The concentration trends seen for C_2Cl_4 in needles sampled at Devilla forest are representative of the typical patterns seen for the other compounds. **Figure 6.1.6(a-c)** shows the ng/g profiles over time for the two needles classes at the 13m and 10m heights for 3 sampling occasions. Note the particularly large variation in the blank analyses for the May sampling.



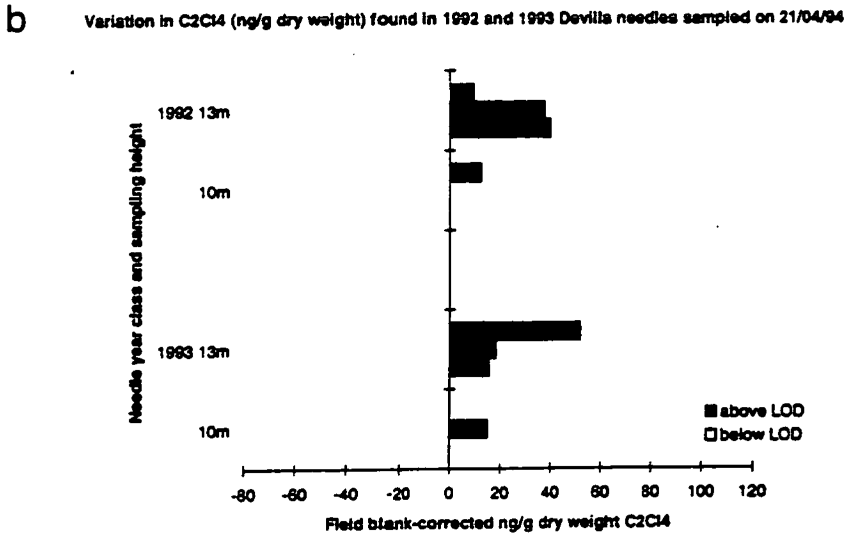


Figure 6.1.6(a-c) ng/g plots for C_2Cl_4 in Devilla needles for 3 sampling occasions.

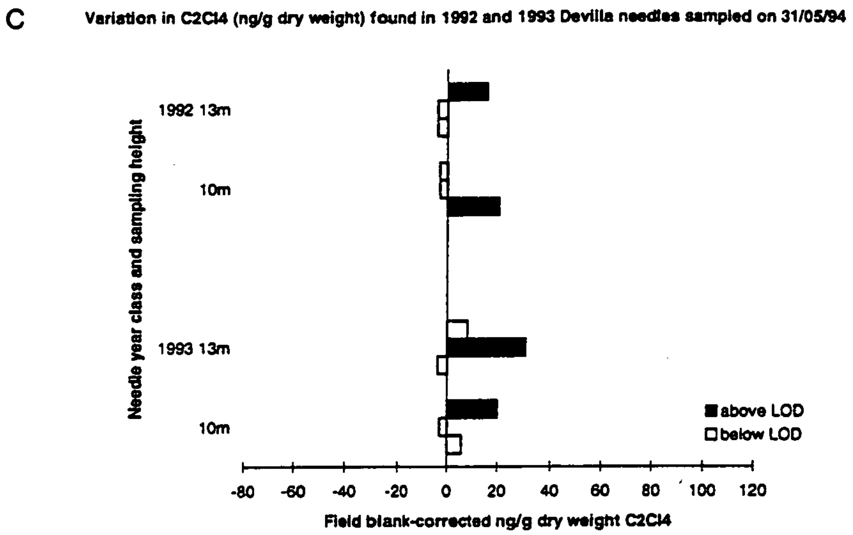


Figure 6.1.6(a-c) The ng/g plots for C_2Cl_4 in Devilla needles for 3 sampling occasions

As a final example from Devilla forest, a ng/g profile for CCl_4 concentration in 1993 needles is presented in **Figure 6.1.7**.

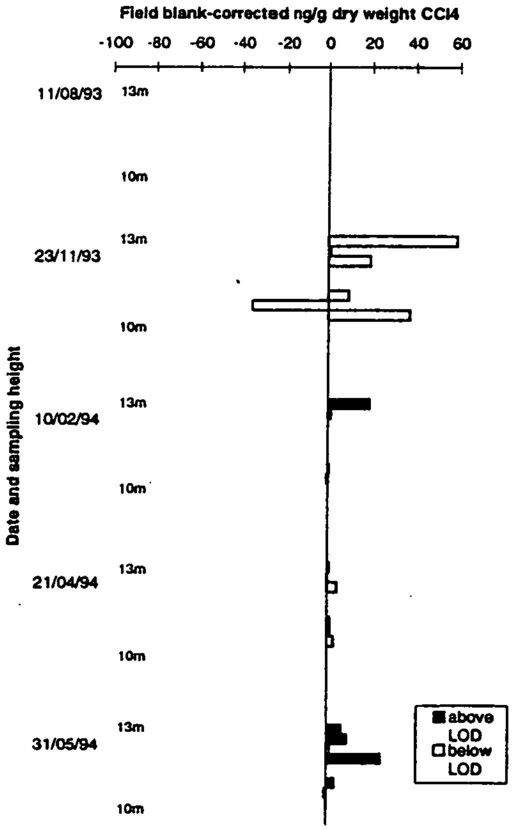


Figure 6.1.7 The ng/g profile for CCl_4 concentration in 1993 needles at Devilla forest.

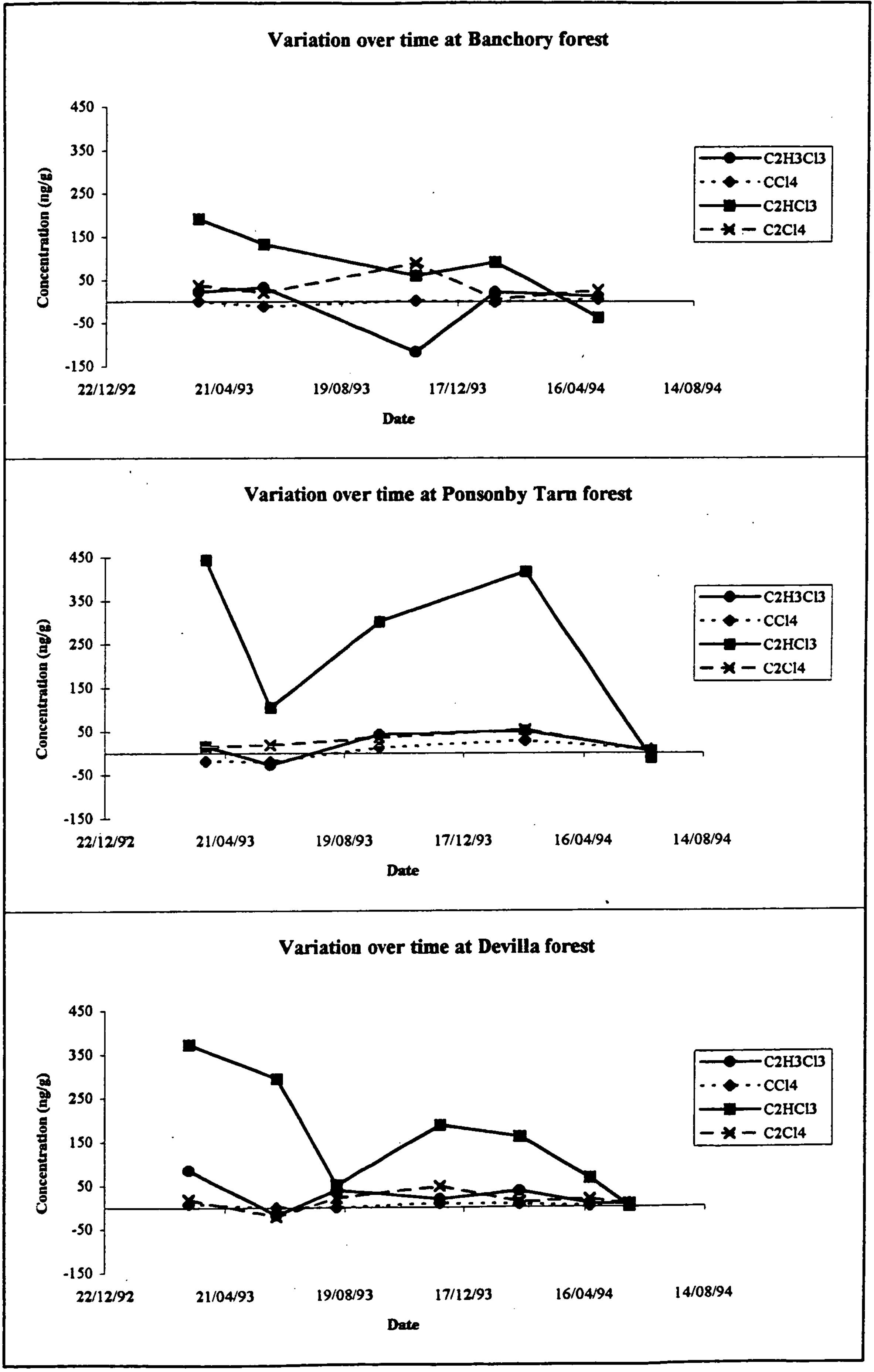
6.2 Seasonal variation

Figure 6.2.1. shows the mean (of triplicate analyses) value variation over the year for each compound at each forest. Data for each of the individual compounds are shown in **Figures 6.2.2-5**. The values found to be significantly above the LOD at the 95% level are given by open symbols (refer to Tables 6.4.1.2-4 containing ANOVA and t-test summaries).

During the year, the highest levels of all the compounds were obtained in the winter season. This could be interpreted in terms of reduced atmospheric degradation reactions and reduced air exchange in the atmosphere due to temperature inversions (Plümacher and Renner, 1991). Similar results have been reported by Plümacher and Renner (1993) for the same chlorinated solvents in pine needles.

Overall the variation was not marked (except for C_2HCl_3), however, as the sampling intervals were too infrequent. Except for new needles, variation with season for wax composition is not generally observed for conifers (Günthardt - Goerg, 1986). After emergence, the epicuticular wax of new needles changes rapidly both in amount and composition with crystalloids developing during the year (Gülaz and Boor, 1992). Therefore the main presumed sink for the chlorinated solvents in pine needles is unlikely to vary greatly and be a source of systematic seasonal variation.

FIGURE 6.2.1



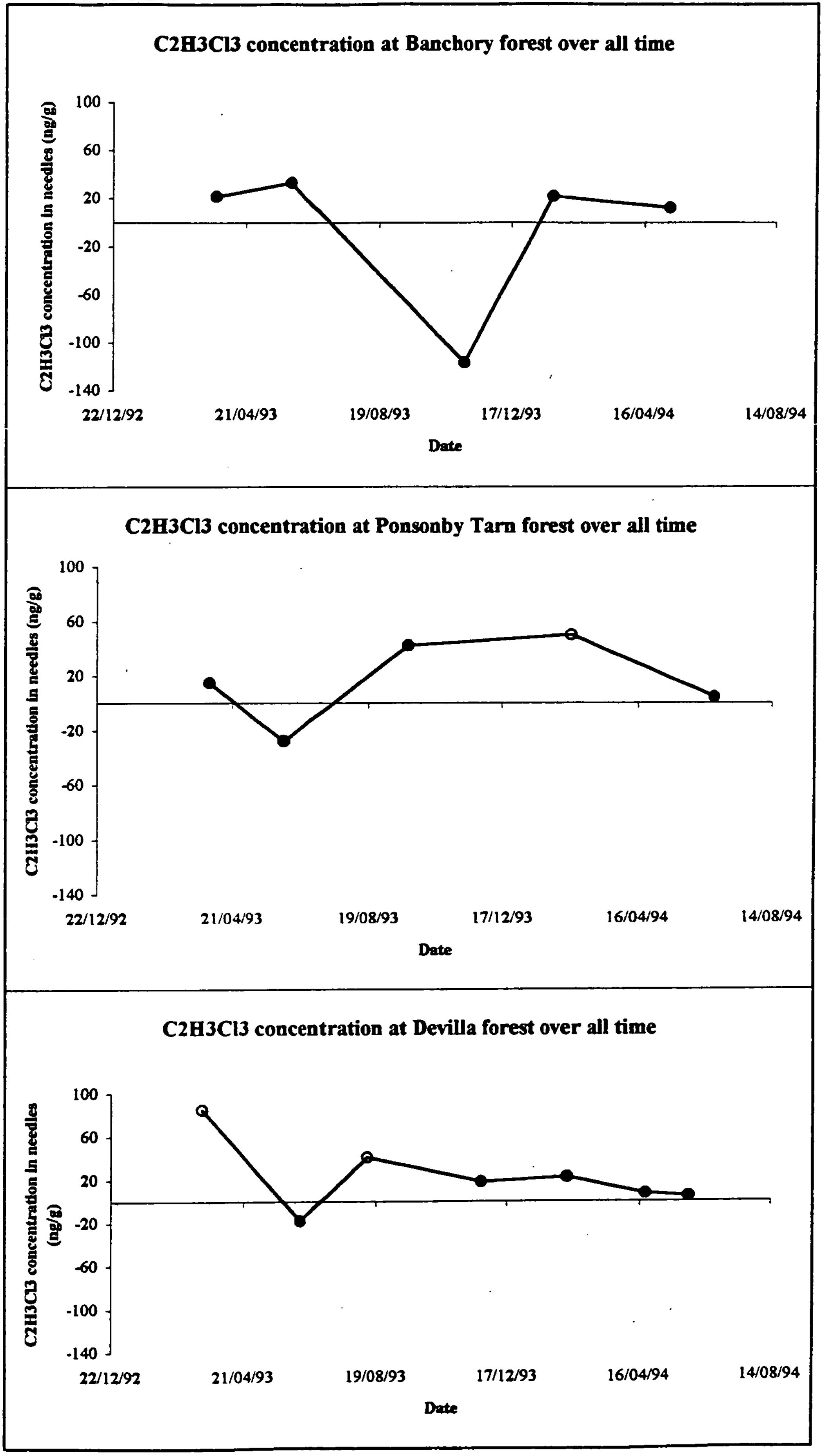
Variation over time at Banchory forest

Variation over time at Ponsonby Tarn forest

Variation over time at Devilla forest

Figure 6.2.1 Variation of the mean concentration for each compound over time at each forest.

FIGURE 6.2.2



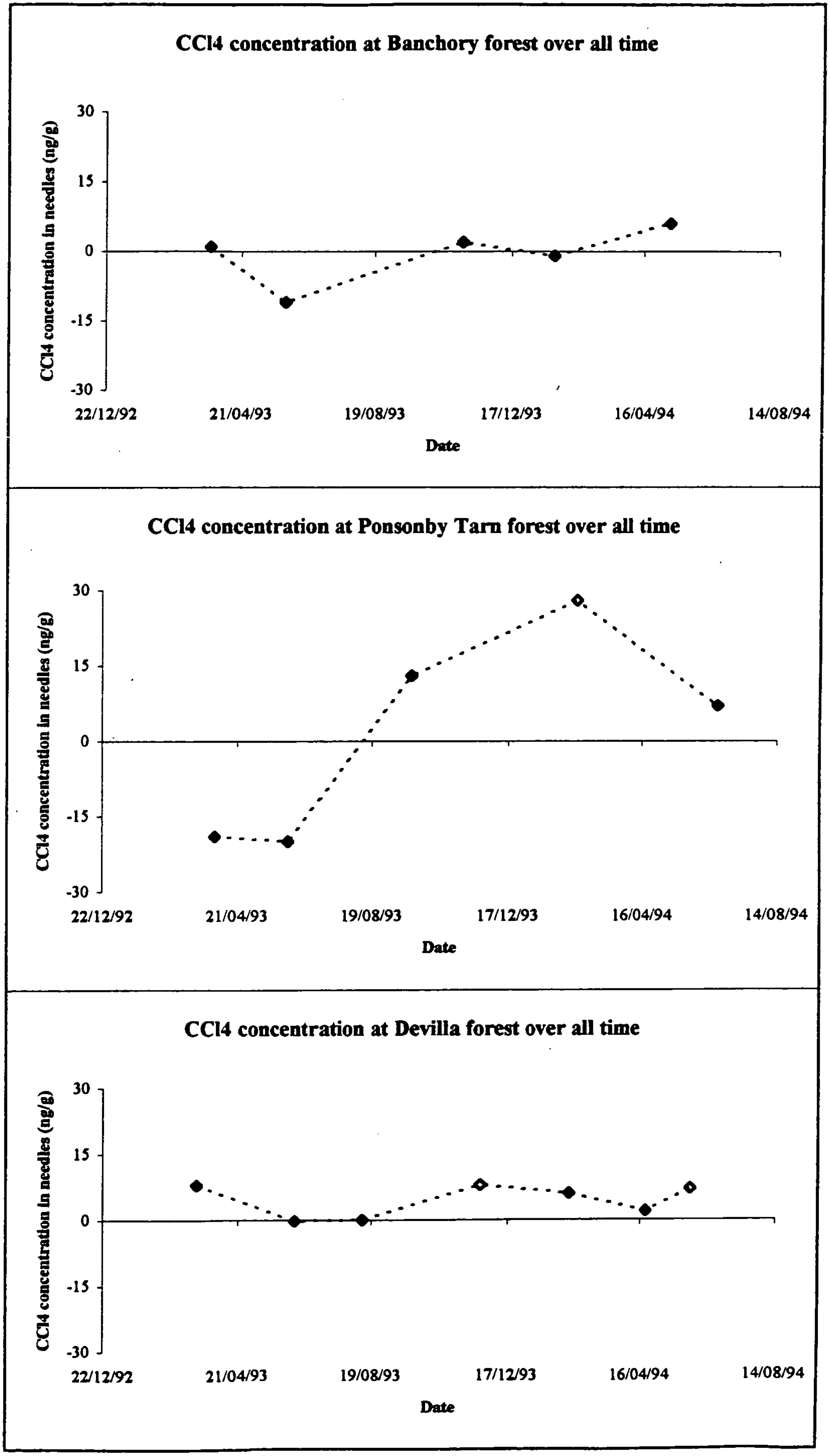
C2H3Cl3 concentration at Banchory forest over all time

C2H3Cl3 concentration at Ponsonby Tarn forest over all time

C2H3Cl3 concentration at Devilla forest over all time

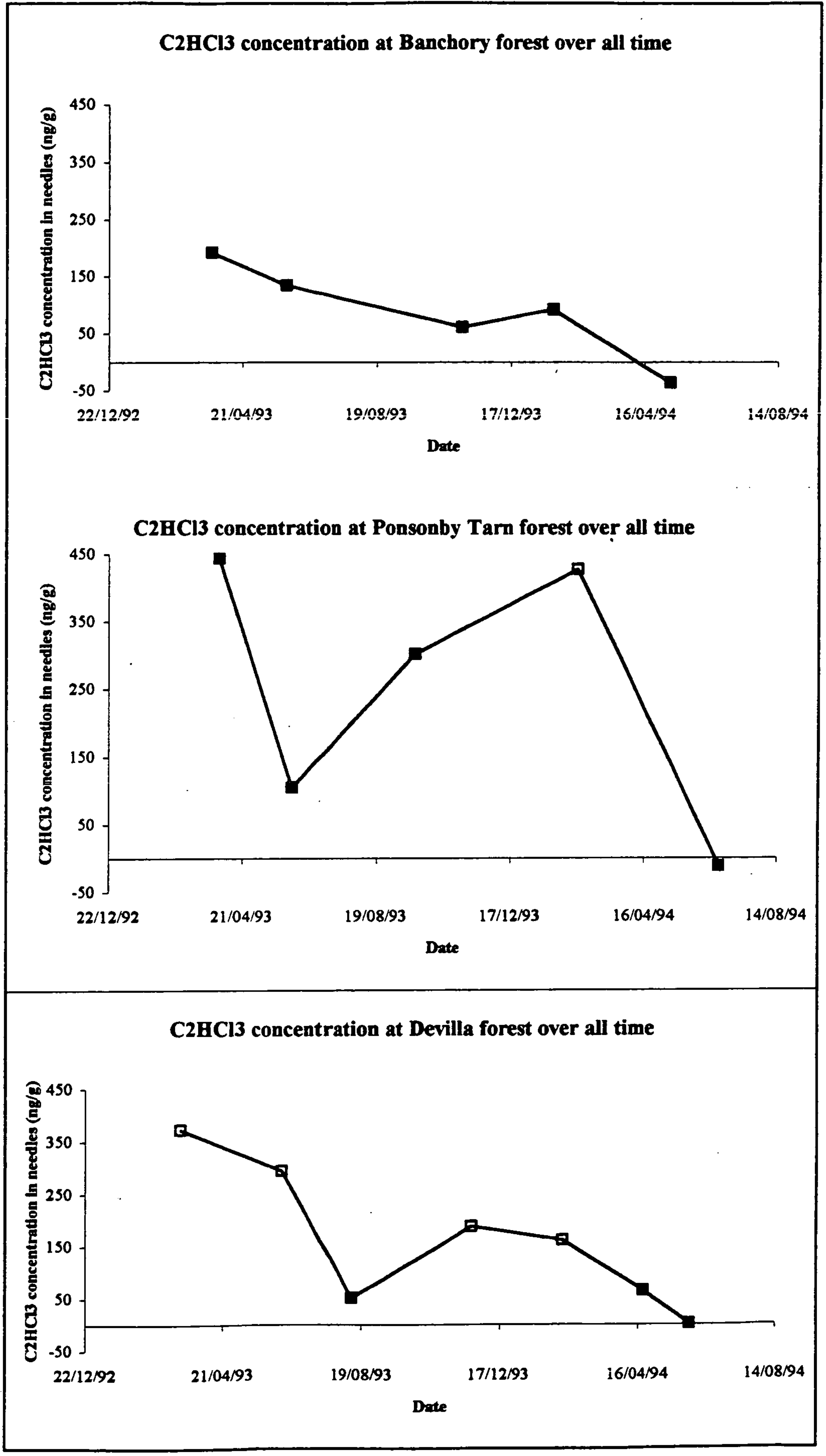
Significant (95% confidence level) data shown as open symbols

FIGURE 6.2.3



Significant (95% confidence level) data shown as open symbols

FIGURE 6.2.4

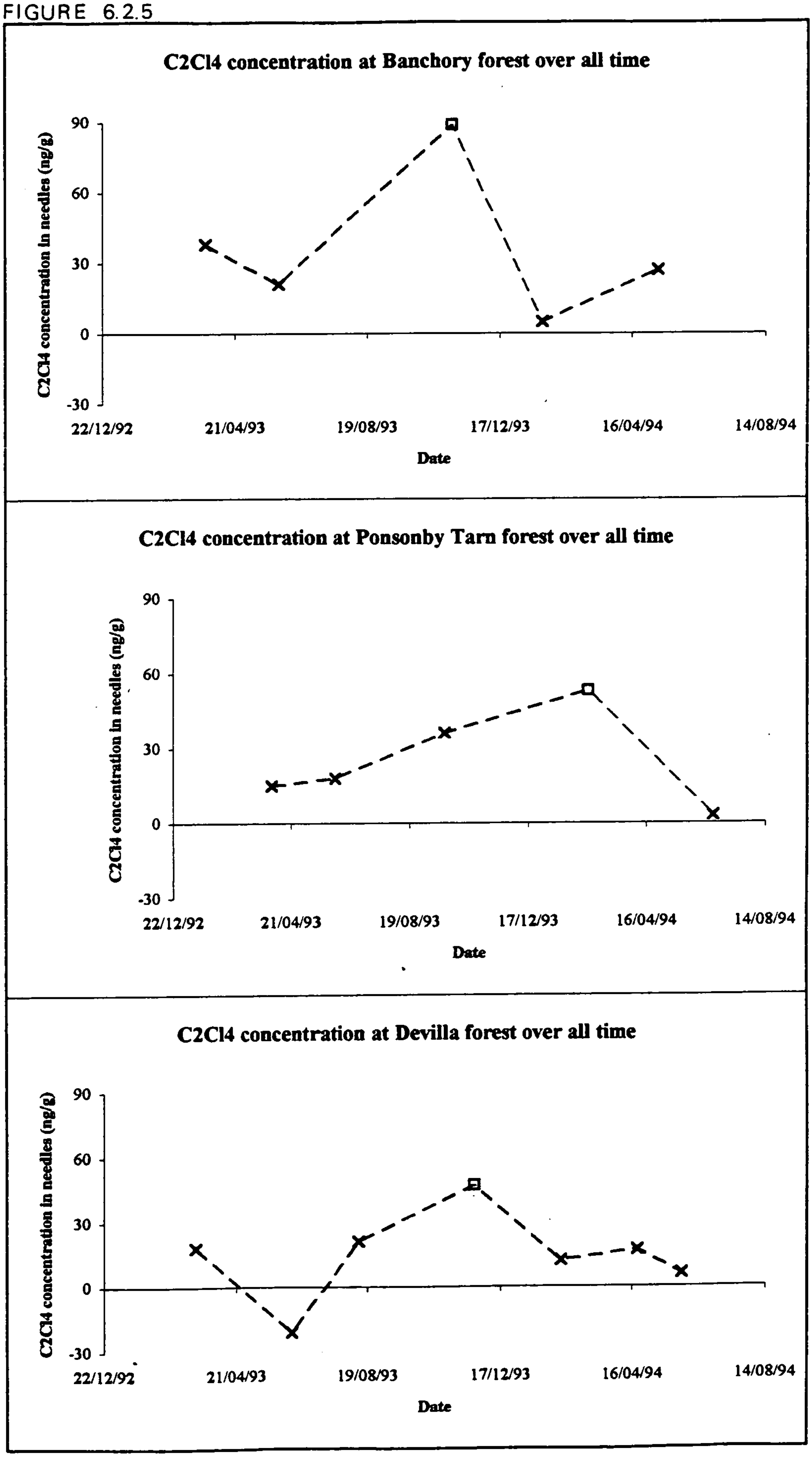

C2HCl3 concentration at Banchory forest over all time

C2HCl3 concentration at Ponsonby Tarn forest over all time

C2HCl3 concentration at Devilla forest over all time

Significant (95% confidence level) data shown as open symbols

FIGURE 6.2.5



Significant (95% confidence level) data shown as open symbols

6.3 Comparison with previous data

The chlorinated solvent concentrations found in pine needles from the three forests in northern Britain are generally larger than those seen in needles from other coniferous forests in Europe (Diezel *et al.*, 1988; Frank and Frank, 1989; Plümacher and Renner, 1993). The typical geometric means seen at the British forests for the four compounds for which reliable quantitative data were collected ($C_2H_3Cl_3$, CCl_4, C_2HCl_3 and C_2Cl_4) are shown in Table 6.1.4. Direct comparison with reported data from other groups is not possible due to differences in reporting units and/or tree species (**Table 6.3.1**). However, assuming that data expressed as ng/g dry weight is approximately twice that of results in ng/g fresh weight, it can be seen that for certain sampling occasions, the mean amounts of the chlorocarbons are considerably greater in the Scots pine forests of Britain than in coniferous forests of central Europe. In addition, although the probability density function can be approximated by a log normal distribution (Figure 5.7.1), extremely high or low values occur. The range of the highest and lowest concentrations is particularly marked for CCl_4 and C_2HCl_3, with that of $C_2H_3Cl_3$ at Devilla forest also being high (Table 6.1.3). Plümacher and Renner (1993) report similar extremes (**Table 6.3.1**).

The same general trend of higher concentrations in urban/industrial sites than rural/mountainous can be seen amongst both the British and the central European results. Overall, the amounts of $C_2H_3Cl_3$, CCl_4 and C_2Cl_4 present in Banchory forest (rural) are similar to that of rural/mountainous regions of central Europe. The results for these three compounds from the British forests close to industry tend to be higher than both Banchory forest (though not at the 95% level) and their central European counterparts. The extremes of the reported data from other countries lie within the range observed in Britain. The behaviour of C_2HCl_3 in Britain is completely different than in central Europe, with much higher concentrations at both the rural/mountainous and industrial/urban forests.

Table 6.3.1 Concentrations of chlorocarbons in needles found (1) in urban/industrial regions and (2) in mountainous/rural areas of central Europe.

Compound	Species of tree	Units	Minimum / maximum	Monthly mean	Location	Reference
$C_2H_3Cl_3$	pine	ng/g $_{\text{fresh weight}}$	0.03-1.0	0.1-0.6	Berlin	Plümacher and Renner (1993)
CCl_4			0.01-1.6	0.03-0.4		
C_2HCl_3			<0.05	-		
C_2Cl_4			0.06-35	0.5-6		
$C_2H_3Cl_3$	spruce	ng/g $_{\text{fresh weight}}$		5.5	Industrial (in direction of wind)	Diezel *et al.* (1988)
CCl_4				<3.0		
C_2HCl_3				4.2		
C_2Cl_4				5.5		
$C_2H_3Cl_3$	spruce	ng/g $_{\text{fresh weight}}$		3.5	Industrial (not in direction of wind)	Diezel *et al.* (1988)
CCl_4				5.0		
C_2HCl_3				<3.0		
C_2Cl_4				-		
C_2Cl_4	spruce	ng/cm^3		2.2-4.0	Tübingen (Germany)	Frank and Frank (1989)
$C_2H_3Cl_3$	spruce	ng/g $_{\text{fresh weight}}$	0.1-0.5		Achenkirch (Austria) mountain	Plümacher and Schroder (1993)
CCl_4			1.5-2.0			
C_2HCl_3			0.25-0.5			
C_2Cl_4			0.8-2.3			
C_2Cl_4	pine spruce	ng/g $_{\text{fresh weight}}$		6.0	rural	Smidt (1992)
C_2Cl_4	spruce	ng/cm^3		0.2-2.0	Black forest	Frank and Frank (1989)
$C_2H_3Cl_3$	spruce	ng/g $_{\text{fresh weight}}$		<3.0	Hohe Mark clean air	Diezel *et al.* (1988)
CCl_4				<3.0		
C_2HCl_3				<3.0		
C_2Cl_4				<3.0		

The effect of tree species on concentration is marked; one study reported that remarkable differences appeared between C_2Cl_4 concentrations in needles sampled at the same time in spruce (4.6 ng/g fresh weight), pine (8.3 ng/g fresh weight) and fir (27.0 ng/g fresh weight) trees at the same location (Plümacher and Renner, 1993). The differences observed may reflect the differences in structure and composition of the epicuticular wax, the partitioning of chlorinated solvents between needles of a particular tree species and the gas phase being based on enrichment in the wax.

6.4 Differences within and between forests

6.4.1 Significance testing

As described, the needle data were plotted in pg/injection and ng/g against time, height or distance as appropriate. This allowed visual inspection of trends, with a distinction being made between that data above, and that below, the LOD. Data classed as artefacts (refer to Chapter 2) were ignored for the purposes of plotting and statistical tests. Two-way analysis of variance (ANOVA) tests on the log geometric mean values with needle year class, height, distance, date of sampling and site as factors were then used to detect whether any significant differences existed. Two-tailed t-tests (paired and unpaired) and population t-tests identified the sources of the significant differences. The 95% confidence level was used in all cases.

The population t-test was used to compare a calculated value of t with $t_{0.05,(n1+n2-2)}$ for significance at the 95% level as the underlying variances were assumed to be similar:

$$t_{calculated} = \frac{m_1 - m_2}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Where $m_1 \pm s_1$ = mean of data set 1 ± standard deviation (geometric)

$m_2 \pm s_2$ = mean of data set 2 ± standard deviation (geometric)

n_1 = number of measurements in set 1

n_2 = number of measurements in set 2

The $\log_e$ geometric mean concentrations used for the population t-tests are tabulated by year, site and date in **Table 6.4.1.1**.

Table 6.4.1.1 Log$_e$ geometric mean concentrations (ng/g $_{\text{dry weight}}$) of chlorocarbons in pine needles, calculated using the probability plot method, for sampling occasions where enough data existed. The 1992 and 1993 year data were combined, as was the between site data at Ponsonby and Devilla forests when appropriate.

	Banchory forest		**Ponsonby Tarn forest**				**Devilla forest**			
Compound	**Date**	**log$_e$ geometric mean**	**Date**	**west**	**central**	**east**	**Date**	**10m**		**13m**
$C_2H_3Cl_3$	24/01/94	3.2±0.7	01/04/93		2.3±2.1		15/03/93		4.2±1.6	
	09/05/94	2.5±1.2	23/09/93		1.4±3.7		11/08/93		4.1±0.2	
			16/02/94	3.5±0.9	4.6±0.2	3.7±0.7	10/02/94	1.8±1.6		2.5±3.0
							21/04/94		2.5±0.9	
										2.8±1.2
							31/05/94		1.8±1.4	
CCl_4	04/11/93	1.4±2.8	16/02/94	2.5±0.9	3.9±1.2	3.2±0.5	15/03/93		2.1±0.7	
	09/05/94	2.1±0.2	23/05/94		0.5±2.1		11/06/93		0.9±1.4	
							23/11/93	2.3±1.4		3.9±0.7
							10/02/94	1.8±1.2		1.6±1.4
							21/04/94	1.8±1.2		1.2±0.7
							31/05/94	1.2±0.7		2.5±0.7
C_2HCl_3	04/11/93	4.4±0.5	01/04/93		6.0±0.9		15/03/93		6.0±0.2	
	24/01/94	4.6±0.9	23/09/93		5.8±0.5		11/06/93		5.8±0.9	
			16/02/94	6.2±0.5	6.2±1.2	6.5±0.2	11/08/93	3.5±0.7		4.8±0.9
							23/11/93	4.8±0.9		5.8±0.5
							10/02/94	4.6±0.9		5.5±0.5
							21/04/94	3.2±3.8		4.8±0.7
C_2Cl_4	04/11/93	4.6±0.2	01/04/93		2.8±1.4		15/03/93		3.0±0.7	
	24/01/94	1.6±1.6	23/09/93		3.0±1.8		11/08/93		2.3±1.8	
										2.9±1.7
	09/06/94	2.8±2.1	16/02/94	3.7±1.8	4.4±0.7	3.5±1.2	23/11/93	3.0±1.2		4.8±1.2
							10/02/94	2.5±0.2		2.5±1.2
							21/04/94	2.8±0.9		3.5±1.2
							31/05/94		2.1±1.2	

The results from the ANOVA and t-tests performed on the log (ng/g dry weight) needle data to establish whether significant differences exist at the 95% confidence level are summarised in **Tables 6.4.1.2-4**.

Tables 6.4.1.2-4 ANOVA and t-test summaries. Significance is at the 95% level (√). Population t-test results that are significant are in **bold**. Dates corresponding to the high concentration which results in the significant difference seen are also shown in **bold**. The needle year classes are 1992 and 1993. The population t-tests are limited to those where enough data was available to calculate the $\log_e$ geometric mean concentration.

Table 6.4.1.2 ANOVA and t-test summaries for Banchory forest data. Only data from the sampling dates 04/11/93, 24/01/94 and 09/05/94 were used (i.e. after 1993 needle growth).

	ANOVA with Year class and Date as factors		**Paired two tailed t-test** for Year class differences	**Population t-test on $\log_e$ geometric mean values** for significant differences between Dates	
$C_2H_3Cl_3$	x	√	x	1.6	24/01/94-09/05/94
CCl_4	x	x	x	0.7	04/11/93-09/05/94
C_2HCl_3	x	√	x	-0.5	04/11/93-24/01/94
C_2Cl_4	x	√	x	**4.6**	**04/11/93**-24/01/94
				-0.9	24/01/94-09/06/94
				2.2	**04/11/93**-09/06/94

The results from the ANOVA and paired t-test in the Table above for Banchory forest show that there is no significant difference between concentrations in needles of the 1992 and 1993 year classes. The significant differences between concentrations of C_2Cl_4 on different dates arise from high values on 04/11/93. The population t-tests did not identify any significant differences in concentrations of the other compounds on the dates tested for.

Table 6.4.1.3 ANOVA and t-test summaries for Ponsonby Tarn forest data. Only data from the sampling dates 16/02/94 and 23/05/94 for the ANOVA by date and site were used. Significantly different results from the unpaired t-test not seen as such by the population t-test are in brackets.

w=west c=central e=east	**ANOVA** with Year & Site as factors		**ANOVA** with Date and Site as factors		**Unpaired two tailed t-test** for Site differences			**Population t-test on $\log_e$ geometric mean values** for significant differences between Dates and Sites			
					w-c	w-e	c-e	w-c	w-e	c-e	
$C_2H_3Cl_3$	x	√	√	√	√	x	√	**-3.0**	-0.5	**3.1**	**16/02/94**
					c>w		c>e		0.5		01/04/93-23/09/93
								-1.3	**-2.2**	-1.4	23/09/93-**16/02/94**
								(w)	(c)	(e)	
CCl_4	x	√	√	x	√	x	x	**-2.2**	-1.5	1.2	**16/02/94**
					c>w			**-2.4**	**-3.7**	**-3.1**	23/05/94-**16/02/94**
								(w)	(c)	(e)	
C_2HCl_3	x	x	√	x	x	x	x	0	-1.5	-0.5	16/02/94
									0.6		01/04/93-23/09/93
								1.9		**-3.4**	23/09/93-**16/02/94**
								(w)		(e)	
C_2Cl_4	x	√	√	x	√	x	√	-1.0	-0.2	1.6	16/02/94
					(c>w)		(c>e)		-0.2		01/04/93-23/09/93
								-0.7	-1.8	-0.9	23/09/93-16/02/94
								(w)	(c)	(e)	
								-0.8	**-2.5**	-0.9	01/04/94-**16/02/94**
								(w)	(c)	(e)	

Table 6.4.1.4 ANOVA and t-test summaries for Devilla forest data. Only the significant results are shown for the population t-tests due to the large number of combinations possible.† at the 90% level.

	ANOVA with Year class and Height as factors		**Paired two tailed t-test** for Height differences	**Unpaired two tailed t-test** (combined data) for Height differences	**Unpaired two tailed t-test** for Year class differences	**Population t-test on log$_e$ geometric mean values** for Height differences		**Population t-test on log$_e$ geometric mean values** for significant differences between Dates	
$C_2H_3Cl_3$	√	x	x 1992	x	√			**2.7**	**15/03/93-21/04/94(amal)**
			x 1993					**-3.3**	**15/03/93-31/05/94 (amal)**
								-3.8	**11/08/93-10/02/94 (10m)**
CCl_4	x	√	x 1992	√	x	**2.7**	**23/11/93**	**-5.4**	**23/11/93-15/03/93 (13m)**
			√ 1993†			**3.6**	**31/05/94**	**-4.6**	**23/11/93-15/03/93 (10m)**
								4.8	**23/11/93-11/06/93 (13m)**
								3.9	**23/11/93-10/02/94 (13m)**
								-3.6	**31/05/94-11/06/93 (13m)**
								3.6	**31/05/94-11/06/93 (13m)**
C_2HCl_3	x	√	√ 1992	√	x	**2.9**	**11/08/93**	**3.2**	**15/03/93-10/02/94 (10m)**
			x 1993			**2.2**	**23/11/93**	**2.7**	**15/03/93-11/08/93 (13m)**
						2.2	**10/02/94**	**7.8**	**15/03/93-11/08/93 (10m)**
								3.0	**15/03/93-23/11/93 (13m)**
								5.8	**11/06/93-11/08/93 (10m)**
								2.2	**11/06/93-23/11/93 (10m)**
								2.2	**23/11/93-11/08/93 (13m)**
								2.9	**23/11/93-11/08/93 (10m)**
								-2.7	**23/11/93-10/02/94 (13m)**
								2.3	**10/02/94-21/04/94 (13m)**
C_2Cl_4	x	√	√ 1992	√	x	**2.6**	**23/11/93**	**-2.2**	**23/11/93-11/08/93 (13m)**
			√ 1993					**3.4**	**23/11/93-10/02/94 (13m)**
								-2.5	**23/11/93-21/04/94 (13m)**
								-4.8	**23/11/93-31/05/94 (amal)**

Table 6.4.1.3 shows that significant differences in concentrations exist on different dates arising from high values on 16/02/94 at Ponsonby Tarn forest. Significant differences are also observed between sites, with the central site having largest concentrations. Again, no significant differences in concentrations in the different needle year classes are reported.

The results in **Table 6.4.1.4** from Devilla forest show that while no significant differences are seen between the 1992 and 1993 year class concentrations (except $C_2H_3Cl_3$), significant differences are shown for height, and also between dates. The high concentration on 15/03/93 appears to be of particular importance.

The results from these tables are discussed in more detail in the next section.

6.4.2 Spatial and temporal differences within sites

6.4.2.1 Ponsonby Tarn

The hypothesis to be tested in the choice of these sites was that any BNFL operations at Sellafield, which involved use of chlorinated solvents, would be a point source. If so, a gradient of decreasing concentration would be seen from west to east. Visual inspection revealed that all four compounds displayed a similar pattern over time. Concentrations at the central site were consistently higher than those of the western or eastern sites for the majority of sampling occasions (Figures 6.1.2-4; Table 6.1.2). The western and eastern sites generally contained similar amounts. The ANOVA results (by site) confirmed that significant differences between sites existed for $C_2H_3Cl_3$, CCl_4 and C_2Cl_4; the two-tailed t-test identified the source of the significance (Table 6.4.1.3). For these three solvents, where a significant difference between sites is identified, the concentration at the central site is greater than that of the western or eastern site ($C_2H_3Cl_3$ at the 90% level). The hypothesised decrease in concentrations from west to east was not seen as a prominent trend.

At least one source of concentrations significantly greater than LOD values for $C_2H_3Cl_3$ and CCl_4 can be identified by the population t-tests as the particularly high concentrations on 16/02/94 (Table 6.4.1.3). The source of the significance for C_2Cl_4 could not be identified as log geometric mean values for all the dates could not be done.

Although C_2HCl_3 shows similar trends to the other compounds upon visual inspection (Figure 6.1.4), the ANOVA (by site and year class) and two-tailed t-tests show that no significant differences between concentrations exist. The population t-tests confirm this, although they are limited in their usefulness by only being able to be applied to sampling occasions where enough positive data exists to calculate a log geometric mean. A date specific ANOVA (16/02/94-23/05/94) does show significance, hence the open symbol for 16/02/94 in Figure 6.2.4. It is interesting to note that while the highest concentrations of C_2HCl_3 are seen at Ponsonby Tarn, and this chlorocarbon quantitatively plays the dominant role amongst all the compounds measured in each of the forests, the wide variation in measured concentrations results in few of the monthly concentrations being statistically significant even when values appear to be very high. There is no obvious reason why the results for C_2HCl_3 from the British forests are so much greater than those found in central Europe (Table 6.3.1). Wide fluctuations in air concentrations across Europe have been reported depending on meteorological conditions and location of monitoring site relative to emission source (Frank, 1991). It would be expected that correspondingly large fluctuations in amounts would also be seen in the needles.

Relationship between concentration and wind speed/direction

The wind direction, and available data on wind speed, for the week preceding each of the needle sampling occasions at Ponsonby Tarn forest are summarised in **Table 6.4.2.1.1**. The Table shows that the wind speed was predominantly light to moderate (2.0-10.0 m/sec) for the period preceding sampling. The wind direction was generally from the SE-S. These observations are representative of wind direction and speed in this part of West Cumbria.

For the particular sampling date identified as having significantly larger concentrations (16/02/94), the wind speed was moderate (i.e. moderate dilution, refer to Chapter 1), but the wind direction contained a larger easterly component than usual. The Sellafield site is due west of the forest sampling sites (Chapter 2) and is not therefore implicated as a source of any significance on this particular sampling occasion assuming wind conditions were not due to air mass inversions.

All the evidence from visual inspection, statistical testing and wind data supports the conclusion that Sellafield is not acting as a significant point source of these chlorinated solvents in Ponsonby Tarn forest needles during the weeks preceding the three monthly sampling occasions. The use of these compounds by the factory is

Table 6.4.2.1.1 Wind direction and speeds measured at a height of 3m at Peterburgh Farm, 3km Northwest of Ponsonby Tarn forest. Dates for which high concentrations are significant at the 95% level are present are shown in **bold**.

Sampling date *(significant compounds)*	**General wind direction**	**Wind speed†** (% of time)
01/04/93	SE-SW	vl 11% l 33% m 47% s 10%
05/06/93	SE-SW	vl 26% l 46% m 24% s 7%
23/09/93	S-SE	vl 24% l 39% m 42% s 0%
16/02/94 ($C_2H_3Cl_3$, CCl_4, C_2HCl_3)	E-SE	vl 11% l 33% m 55% s 2%
23/05/94	SE-SW variable	vl 85% l 15% m 0% s 0%

†Wind speed

vl = very light (0.5-2.0 m/s)
l = light (2.0-5.0 m/s)
m = moderate (5.0-10.0 m/s)
s = strong (10.0-11.4 m/s)

limited to small-scale use as degreasants and laboratory solvents. Such substances are not used in large quantities in the chemical processing plants (Hall, 1995).Other factors obviously contribute to the generally high (although not significantly different from the other two forests at the 95% level, Figure 6.1.1) chlorinated solvent levels seen for all the compounds at Ponsonby forest .Of the three sites, the central site consistently displays the highest concentrations, which in turn affects the overall mean and median values. This site is the most exposed of all the sites at the three forests, being at a higher elevation than most (70m compared to 40-60m) and surrounded by fewer trees. The trees were the oldest and highest sampled at any forest. Any or all of these factors may contribute to the ability of the needles to 'trap' airborne chemicals.

Conclusions

(1) Needles from the central site at Ponsonby Tarn forest contain significantly higher (at the 95% confidence level) chlorocarbon concentrations than those collected from the western or eastern site.

(2) Significantly higher concentrations of $C_2H_3Cl_3$, CCl_4 and C_2Cl_4 are seen in needles collected on 16/02/94 when the wind direction was easterly during the preceding week. Variations in C_2HCl_3 concentrations over time were not significant at the 95% level.

(3) Sellafield is not implicated as a significant point source of the chlorocarbons detected in the needles sampled on 5 separate occasions at three monthly intervals from Ponsonby Tarn forest. The factory is not ruled out as an occasional or intermittent source.

(4) No other obvious point source which would account for the chlorocarbon concentrations found in the needles can be identified. Other factors such as tree height and exposure to wind may be important, particularly at the central sampling site.

(5) No statistically significant difference exists between concentrations found in 1992 and 1993 year class needles.

6.4.2.2 Banchory

Visual inspection of the ng/g plots (Figure 6.1.5(a,b)) reveals that concentrations remain reasonably constant over time. Referring to the statistical results (Table 6.4.1.2), the sampling date is shown to be significant within the 04/11/93 : 24/01/94 : 09/05/94 set. The population t-tests are particularly limited for Banchory as many median calculations are not possible due to the size of the data set. However, the date source for the significant difference for C_2Cl_4 was identified as being the high concentration found on 04/11/93. The source of the significance was not identified for $C_2H_3Cl_3$ or C_2HCl_3. No significance was shown for CCl_4. Similar concentrations are seen for all the compounds at Banchory forest, with C_2HCl_3 being the highest.

Relationship between concentration and wind speed/direction

The wind data for the week preceding each sampling occasion are summarised in **Table 6.4.2.2.1**. The wind speed and direction were measured at Mannofield on the outskirts of Aberdeen (20 km north east of the sampling site). As can be seen from the Table, the wind direction is predominantly from the S-SW, with windspeeds generally very light to light (0.5-5.0 m/s). The similarity of the wind patterns displayed during the sampling period to those observed at Ponsonby and Devilla forests make Banchory a very suitable 'rural' forest to be used for comparison purposes.

Table 6.4.2.2.1 Wind speed and direction measured at Mannofield, 20 km north east of Banchory forest, for the week preceding needle sampling. Dates where significantly high concentrations at the 95% level are present are shown in **bold**.

Sampling date *(significant compounds)*	**General wind direction**	**Wind speed†** (% of time)
23/03/93	SW	vl 0% l 14% m 71% s 14 %
31/05/93	S then N-NE	vl 57% l 14% m 29% s 0%
04/11/93 *(C_2Cl_4)*	SW then variable (N to E to SW)	vl 57% l 43% m 0% s 0%
24/01/94	S-SW	vl 57 % l 14 % m 14 % s 14 %
09/05/94	SW-SE	vl 71% l 29% m 0% s 0%

†Wind speed

vl = very light (0.5-2.0 m/s)
l = light (2.0-5.0 m/s)
m = moderate (5.0-10.0 m/s)
s = strong (10.0-11.4 m/s)

Conclusions

(1) Very little variation in concentration over time was shown for all the compounds.

(2) The predominant wind direction was from the SW with wind speeds usually very light to light (less dispersion). The wind speed and direction are comparable with those seen at the forests located close to industry. The geographical features are similar (nearby coast and low hills).

(3) No statistically significant variation between the 1992 and 1993 year class needles was seen for any of the compounds.

6.4.2.3 Devilla

Visual inspection of Figures 6.1.6-7 reveals the general pattern shown by the concentrations found in Devilla needles. Higher concentrations were often present at the 13m height, particularly for some of the compounds. The results from the ANOVA (and paired/unpaired t-tests) performed with height and year class as factors (Table 6.4.1.4) confirm that significant differences for CCl_4, C_2HCl_3 and C_2Cl_4 by height exist.

$C_2H_3Cl_3$ is the only chlorinated solvent at any forest or site to show a significant concentration difference for needle year class. This result may have arisen by chance out of the number of combinations made, i.e., at the 95% level, one result out of 20 can be ascribed to chance. Population t-tests by height did combine the year class data to identify the source of the significance (**Table 6.4.2.3.1**), with three dates identified for C_2HCl_3, two for CCl_4 and one for C_2Cl_4. Only one sampling date (23/11/93) was significant by height for all three compounds. Non-significant differences were recorded for other dates when there was sufficient data for the median calculation. The significance of the 23/11/93 sampling for C_2Cl_4 is clearly seen from Figure 6.1.6a as are the greater amounts at the 13m height.

Table 6.4.2.3.1 Significance by height using population t-tests on $\log_e$ geometric mean concentrations calculated for some sampling dates.

Compound	Date when significant difference observed	Pattern of difference
$C_2H_3Cl_3$	11/08/93	13m>10m
	23/11/93	13m>10m
	10/02/94	13m>10m
CCl_4	23/11/93	13m>10m
	31/05/94	13m>10m
C_2HCl_3		
C_2Cl_4	23/11/93	13m>10m

For all the dates where a significant difference in concentrations between heights was recorded, the concentration at 13m was greater than that at 10m; i.e. 6 out of 11 comparisons made.

Population t-tests by date confirmed the significance of certain sampling occasions (Table 6.4.1.4). All the sampling dates except one (21/04/94) were found to be significant for at least one compound.

The highest concentrations found at Devilla forest were again those of C_2HCl_3, which, as in Ponsonby and Banchory forests, does not show many significant differences between the concentrations found, indicating that there is much variation about the mean.

Relationship between concentration and wind speed/direction

The wind speed at the canopy top is predominantly light to moderate (2.0-10.0 m/s), and generally from the south west (**Table 6.4.2.3.2**). The higher percentage of greater windspeeds than at the other two sites indicates that the dispersion along the predominant wind direction is likely to be greater.

The combined evidence from visual inspection, statistical testing and wind data suggests that the petrochemical industry at Grangemouth, located 7km to the south of the forest, could be a point source of chlorinated solvents. The predominant wind direction is from the south, and a vertical profile through the canopy is confirmed, as would be expected if airborne plumes of pollutants were regularly transported across the forest during conditions where mixing into the canopy is supressed (i.e. low wind speed).

Table 6.4.2.3.2 Wind speed and direction measured at Devilla forest sampling site from a height of 15m, or at Kings Buildings (University of Edinburgh) at a height of 20m, for the week preceding needle sampling. Dates where significantly high concentrations at the 95% level are present are shown in **bold**.

Sampling date *(significant compounds)*	**General wind direction**	**Wind speed†** (% of time)
15/03/93[$] *($C_2H_3Cl_3$, C_2HCl_3)*	SW variable then SE-NE 2 days SW variable	vl 7% l 20% m 40% s 33%
11/06/93[‡] *(C_2HCl_3)*	SW then E	vl 36% l 64% m 0% s 0%
11/08/93[‡] *($C_2H_3Cl_3$)*	WSW then E	vl 45% l 53% m 2% s 0%
23/11/93[$] *($C_2HCl_3$, CCl_4, C_2Cl_4)*	S-SW variable SE variable then NW-W	vl 40% l 35% m 20% s 5%
10/02/94[$] *($C_2HCl_3$)*	SE-NE SW variable	vl 10% l 12% m 38% s 40%
21/04/94[$]	W-SW variable	vl 20% l 55% m 20% s 5 %
31/05/94[‡] *(CCl_4)*	Variable SW last 2 days	vl 87% l 13% m 0% s 0%

†Wind speed

vl = very light (0.5-2.0 m/s)
l = light (2.0-5.0 m/s)
m = moderate (5.0-10.0 m/s)
s = strong (10.0-11.4 m/s)

[‡] Data provided by J.N.Cape
[$] Data provided by K.J.Weston

Conclusions

(1) The needle concentrations at the 13m height were shown to be significantly greater than those at 10m for CCl_4, $C_2H_3Cl_3$ and C_2Cl_4 for some sampling occasions as indicated by suppressed mixing at low wind speeds. Fewer significant differences in concentration were shown by C_2HCl_3, in agreement with results from Banchory and Ponsonby Tarn forests.

(2) Higher concentrations in the needles were observed when the wind was blowing from the S-SW, i.e. from the direction of the presumed source during the week preceding sampling, although the generally light to moderate winds indicate that dispersal along this wind direction could be important.

(3) Grangemouth is implicated as a possible source of the chlorocarbon concentrations seen at Devilla forest on the dates sampled.

(4) Only $C_2H_3Cl_3$ out of the four compounds shows statistically significant variation between the 1992 and 1993 needle year classes.

6.4.3 Differences between sites

The $\log_e$ geometric mean concentrations estimated across the total population at each forest are shown in Figure 6.1.1. As the 95% confidence intervals overlap, it can be concluded that no significant difference exists at this confidence level between the three forests or between year classes. The similarity of the geometric mean concentrations at the three forests across all time indicates that the sources of pollution are more general than specific. The geometric standard deviation range of

2.0-3.0 is similar to that of other air pollutants (Fowler and Cape, 1982) and suggests an atmospheric source (Table 6.1.4).

The industries considered cannot be pinpointed as definite contributors to the chlorocarbon concentrations observed in the needles on the dates sampled. The evidence presented links Grangemouth to needle loadings in Devilla forest more than it does Sellafield to specific Ponsonby Tarn concentrations. Transport over reasonably long distances is suggested as the relatively high concentrations (as compared to other forests in Europe) appear to have no obvious linked source.

The consistently high concentrations found in rural areas in both this study and others across Europe is cause for concern. The relatively long half lives of these compounds in the atmosphere (e.g. up to 42 years for CCl_4) will ensure that, from whatever source they originate, their presence as gaseous pollutants will remain apparent long after the initial emission.

Chapter 7

Results and discussion : Passive air samplers

'Would you tell me, please, which way I ought to go from here?'
'That depends a good deal on where you want to get to,' said the Cat.
'I don't much care where -' said Alice.
'Then it doesn't matter which way you go,' said the Cat.
'- so long as I get *somewhere*,' Alice added as an explanation.
'Oh, you're sure to do that,' said the Cat, 'If you only walk long enough.'

Alice's adventures in Wonderland, Lewis Carroll

7. RESULTS AND DISCUSSION : PASSIVE AIR SAMPLERS

7.1 Results

The sampler blank-corrected wax data in ng/g_{wax} are presented in **Tables 7.1.1-3**. The values greater than the field blank (LOD) are given in italics, and the mean and standard deviation are in bold (as in Tables 6.1.1-3). Negative values are less than the field blank, and the < values are the ALOD ('non-detection'), as determined by GC integration parameters. The full data set is given in Appendix 9. **Table 7.1.4** shows the $\log_e$ geometric mean and standard deviation, and back-transformed geometric mean and standard deviation, at each site over all time calculated using the method described in Chapter 5.

A large variation in wax concentration was found through the year for all four chlorinated solvents. In general, $C_2H_3Cl_3$ concentrations were greater than those of C_2HCl_3, which in turn were larger than CCl_4 and C_2Cl_4.

The relative concentrations of the different chlorocarbons in air have been measured by other workers to be similar to those observed for needles (Chapter 1 and 6), with C_2HCl_3 and C_2Cl_4 having the highest concentrations (Frank and Frank, 1988a). Statistical frequency distributions at rural sites in Europe show that many chlorocarbons are present in air at higher levels than those typical for truly remote sites (Frank, 1991). Chlorocarbons have also been shown to be transported in rain (Plümacher and Renner, 1993). Annual mean rainwater concentrations in forests are between 3 and 7 ng/L (Plümacher and Renner, 1993). The typical median concentration of airborne chlorocarbons in forested regions is 0.4-1.2 µg/m^3 (Frank and Frank, 1988a).

TABLE 7.1.1

Sampler blank-corrected wax concentrations in ng/g							
mean and standard deviation in bold				*Values above LOD in italics*			
BANCHORY							
OUT	IN	**C2H3Cl3**	**CCl4**	**C2HCl3**	**C2Cl4**	**Foliage co-sampled**	
27/04/93	31/05/93	*1034*	-3	142	<0.07	**31/05/93**	
		172	-12	72	<0.07		
		11	-12	117	*88*		
		1833	-6	62	*11*		
		72	-12	98	<0.07		
		2314	-12	162	*100*		
	mean	**906**	**-9**	190	<0.07		
	sd	**989**	**4**	**120**	<0.07		
				47	<0.07		
					22		
					41		
31/05/93	09/07/93	*83*	-14	-115	<0.07		
		40	29	-115	*99*		
		<0.08	20	147	<0.07		
		<0.08	-14	-115	<0.07		
		89	-5	140	<0.07		
		<0.08	12	2	<0.07		
		141	40	174	<0.07		
		174	26	-115	<0.07		
		66	14	0.3	<0.07		
		68	**9**	**133**	**11**		
			21		**33**		
05/11/93	24/01/94	*1276*	-40	*264*	19	**24/01/94**	
		135	7	-33	49		
		2393	6	26	37		
		20	-67	75	-56		
		40	*78*	101	73		
		201	41	27	-56		
		29	-67	*442*	-56		
		585	6	205	90		
		915	**-4**	**138**	**13**		
			51	**157**	**61**		
24/01/94	03/03/94	*-77*	*22*	<0.2	-74		
		87	<0.02	*40*	117		
		-77	*60*	*9*	-74		
		-77	<0.02	*74*	-74		
		241	<0.02	*20*	-74		
		-77	*44*	<0.2	244		
		4	<0.02	<0.2	-22		
		-77	<0.02	*142*	-74		
		-77	*42*	**32**	-74		
		-14	**19**	**50**	**-12**		
		111	**24**		**115**		
09/05/94	09/06/94	624	*30*	-11	*165*		
		-514	-10	173	-18		
		-514	-39	*276*	-18		
		-514	-14	-35	*111*		
		-350	-24	*321*	-18		
		-208	15	213	-18		
		-514	-16	50	-18		
		-284	-39	92	-18		
		417	-12	61	-18		
			24	**127**	**17**		

TABLE 7.1.2

PONSONBY TARN FOREST

			$C_2H_3Cl_3$			CCl_4			C_2HCl_3			C_2Cl_4		**Foliage co-sampled**	
OUT	**IN**	west	central	east	west	central	east	west	central	east	west	central	east		
01/03/93	01/04/93	-41	-41	-41	-27	-48	*36*	200	*1090*	37	43	28	-31	**01/04/93**	
		233	-41	-41	-41	-48	-48	*371*	-204	-108	-9	16	-31		
		96	-41	58	-98	-16	-27	44	*649*	-22	-31	**22**	21		
		193	**-41**	**-8**	**-55**	**-37**	-13	**205**	**512**	**-31**	**1**	**8**	**-14**		
			0	**57**	**38**	**19**	**44**	**163**	**658**	**73**	**38**		**30**		
05/06/93	05/07/93	-32	-66	-66	-25	0.2	-25	-75	-276	-371	-7	-7	-7		
		-66	-12	-66	1	2	-25	-49	*106*	*173*	-7	-7	-7		
		35	-66	-66	-1	*6*	-25	*91*	*140*	**-99**	*42*	*64*	-7		
		-21	**-48**	**-66**	**-8**	**3**	**-25**	**-11**	**-10**	**385**	**9**	**17**	**-7**		
		51	**31**	**0**	**15**	**3**	**0**	**90**	**231**		**28**	**41**	**0**		
09/08/93	23/09/93	-118	*875*	-118	-34	*93*	*30*	345	*793*	412	21	-10	-58	**23/09/93**	
		-118	*3044*	-118	*66*	-34	*17*	289	208	-83	53	*255*	118		
		-118	*2326*	*648*	-34	*136*	-34	**317**	**500**	263	-58	**122**	-2		
		-118	**2082**	**138**	**-1**	**65**	**4**	**39**	**414**	**197**	**5**	**188**	**19**		
		0	**1105**	**442**	**59**	**89**	**34**			**254**	**57**		**90**		
21/01/94	16/02/94	*1312*	*601*	*1349*	1	*73*	1	40	*933*	*684*	*72*	*32*	*32*	**16/02/94**	
		1039	*940*	*1148*	*16*	*26*	*62*	*510*	*594*	*432*	<0.07	*167*	*95*		
		-103	*2315*	-103	*24*	*135*	*32*	*284*	**763**	**558**	<0.07	**66**	<0.07		
		749	**1285**	**798**	**14**	**78**	**32**	**278**	**261**	**178**	**24**	**89**	**43**		
		751	**908**	**787**	**11**	**54**	**30**	**235**			**42**		**49**		
23/05/94	23/06/94	-29	-29	-29	-56	-33	-22	181	73	437	28	-39	-39		
		-29	81	-29	-32	-45	-32	25	226	111	-39	-39	-39		
		10	-29	-29	-36	-26	-37	263	100	-73	-39	38	-39		
		-16	**7**	**-29**	**-41**	**-35**	**-30**	**156**	**133**	**158**	**-16**	**-13**	**-39**		
		23	**64**	**0**	**13**	**9**	**7**	**121**	**82**	**258**	**39**	**45**	**0**		

TABLE 7.1.3

DEVILLA FOREST																
			C2H3Cl3			CCl4			C2HCl3			C2Cl4		Foliage co-sampled	
OUT	IN	13m	10m	7m	13m	10m	7m	13m	10m	7m	13m	10m	7m		
15/03/93	05/05/93	*161*	<0.08	*326*	*59*	*72*	*46*	-31	266	-113	-88	-88	39		
		196	*492*	*114*	-9	*44*	-9	229	-94	136	18	-23	-88		
	mean	178	*207*	*130*	25	-9	-9	99	86	80	-88	-88	-88		
	sd	25	233	190	48	36	10	183	264	172	-63	-67	-46		
			247	117		41	32				61	38	73		
12/07/93	11/08/93	*1105*	*328*	*1167*	*4*	0.6	-1	-28	-28	-28	123	55	-216	11/08/93	
		2407	*1630*	<0.08	*9*	1.4	-1	*353*	62	*224*	*947*	-146	145		
		1756	*2042*	584	-1	-1	-1	*203*	49	-28	-837	160	103		
		920	1334	826	4	0.4	-1	176	28	66	76	23	11		
			896		6	1.2	0	192	49	146	893	156	198		
11/08/93	06/10/93	565	525	*1627*	-4	-4	-4	-42	-42	53	-607	97	-34		
		533	-554	401	*23*	*39*	-4	57	41	-38	229	-68	63		
		266	562	*1353*	*59*	-4	-4	38	25	-42	*505*	227	105		
		455	178	1127	26	10	-4	18	8	-9	42	85	46		
		164	634	643	32	25	0	62	44	63	579	148	71		
06/10/93	23/11/93	299	*737*	522	-20	-20	-20	360	-107	*978*	*166*	*39*	<0.07	23/11/93	
		295	*1243*	-142	*87*	-20	-11	74	-215	-26	<0.07	<0.07	<0.07		
		-91	*1185*	*977*	*65*	-10	27	-244	*793*	*811*	*65*	<0.07	<0.07		
		168	1066	452	44	-17	-1	63	167	588	73	13	<0.07		
		224	277	563	66	6	25	302	664	638	46	22	0		
23/11/93	10/02/94	*1593*	38	175	<0.02	*9*	<0.02	28	-46	-46	-44	-44	-44	10/02/94	
		480	-41	-41	<0.02	*20*	*10*	*319*	*612*	*314*	*188*	-44	-44		
		2796	-41	-41	*46*	<0.02	7	*533*	*427*	*199*	-44	-44	-44		
		1623	-16	31	16	10	6	293	331	166	34	-44	-44		
		1168	46	125	27	10	6	253	339	184	133	0	0		
21/04/94	31/05/94	<0.08	*51*	<0.08	*12*	-12	-2	-55	46	-55	<0.07	<0.07	<0.07	31/05/94	
		<0.08	<0.08	<0.08	*12*	9	-2	-55	-55	-55	<0.07	<0.07	<0.07		
		609	<0.08	<0.08	12	*16*	*8*	-55	77	-55	*24*	<0.07	<0.07		
		203	17	<0.08	0	4	2	-66	23	-66	8	<0.07	<0.07		
		351	30	0		14	6	0	68	0	14	0	0		
31/05/94	27/06/94	-33	-33	-33	-30	-22	8	-80	4	93	-5	-5	-5		
		-33	*12*	*15*	-0.1	-32	10	186	182	*345*	*24*	*30*	-5		
		-33	-33	-33	-16	-32	21	90	*234*	*512*	-5	-5	-5		
		-33	-18	-17	21	-29	13	66	140	317	6	7	-6		
		0	26	26		6	7	135	121	211	17	20	0		

Table 7.1.4 Chlorinated solvent concentrations (ng/g) in wax at each site over all time expressed as log$_e$ geometric mean and standard deviation, and as back-transformed geometric mean and standard deviation.

Compound	Forest	Log$_e$ geometric mean and standard deviation concentration	Geometric mean and standard deviation
$C_2H_3Cl_3$	**Banchory**	5.1±1.0	$164^x/2.7$
	Ponsonby	5.9±1.2	$365^x/3.3$
	Devilla	5.7±1.4	$299^x/4.1$
CCl_4	**Banchory**	1.9±1.3	$7^x/3.7$
	Ponsonby	1.8±0.8	$6^x/2.2$
	Devilla	2.4±0.9	$11^x/2.5$
C_2HCl_3	**Banchory**	4.5±0.8	$90^x/2.2$
	Ponsonby	5.2±1.0	$181^x/2.7$
	Devilla	4.8±1.0	$122^x/2.7$
C_2Cl_4	**Banchory**	2.7±0.4	$15^x/1.5$
	Ponsonby	2.6±1.5	$14^x/4.5$
	Devilla	3.1±1.0	$22^x/2.7$

7.2 Comparison with concentrations measured in needles

Approximately half the air samplers were collected (after exposure for 1 month) at the same time as needle samples. The (blank-corrected) concentration data from these co-sampled tubes can be directly compared to the corresponding data obtained from the needle analyses. The relationship found between compound concentration in needles and co-sampled air sampler tube wax is shown graphically, for comparison with the partition ratio data determined in the laboratory (Chapter 4).

Air sampler data co-sampled with needles was plotted as ng/$g_{dry\ weight}$ needle (*y* axis) versus ng/g_{wax} (*x* axis) (**Figures 7.2.1-4**). All of the non-constrained data is plotted in the figures (including negative points) to allow visual comparison of differences within and between forests. As the F-test for the intercept showed non-significance at the 95% level, slope and intercept data were also obtained when the regression lines were constrained to go through zero, and when points below zero were excluded (**Table 7.2.1**).

The results summarised in **Figures 7.2.1-4** show that wax concentrations of all the airborne chlorocarbons were proportional to the concentrations found in co-sampled needles. For sampling occasions when large amounts of chlorinated solvent were found in the needles, large amounts were also seen in the wax. The plots show that the wax ad/absorbs from the air greater concentrations on a weight for weight basis than the needles from the air, particularly $C_2H_3Cl_3$. This can be interpreted as the wax (1% of the needle dry weight) being the major sink. Epicuticular wax is a very effective scavenger for lipophiles (Frank and Frank, 1986). The partition ratios reflect the enhanced uptake from the air by the wax over the needles (refer to Table 4.3.3.3).

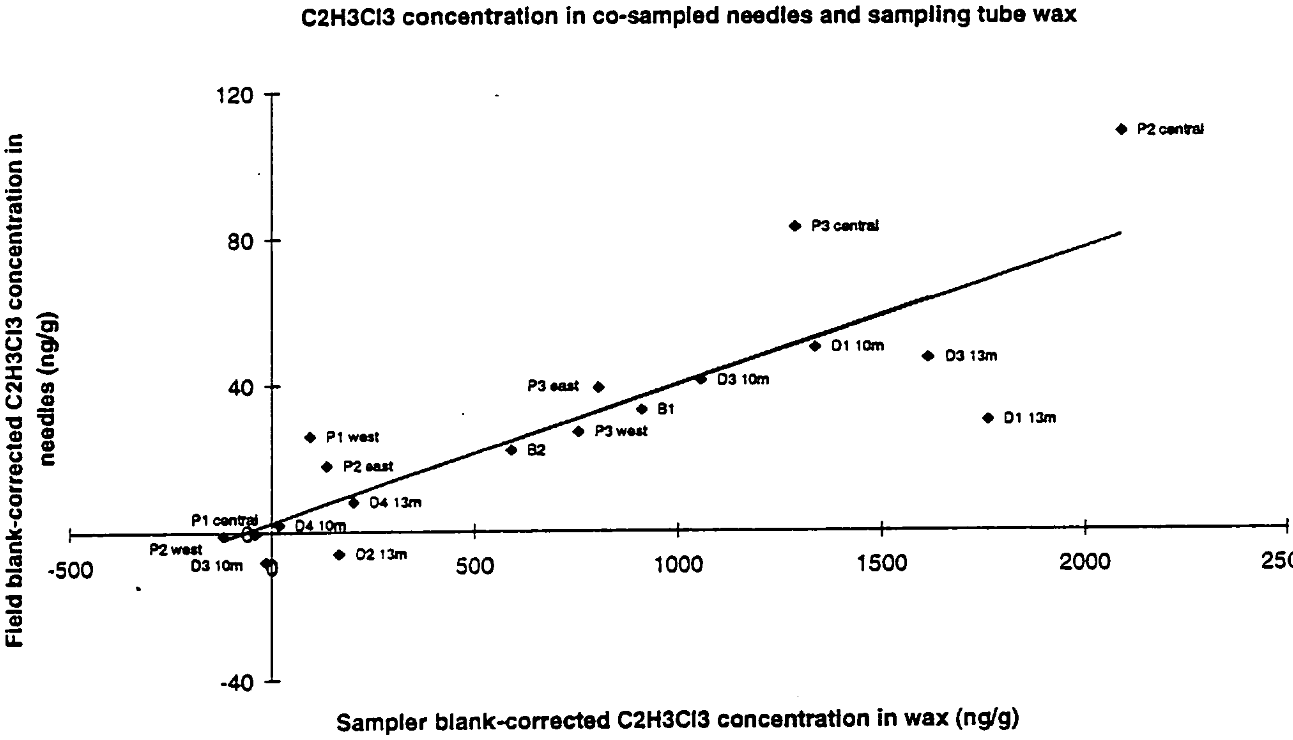


Figure 7.2.1 $C_2H_3Cl_3$ concentration in co-sampled needles and sampling tube wax.

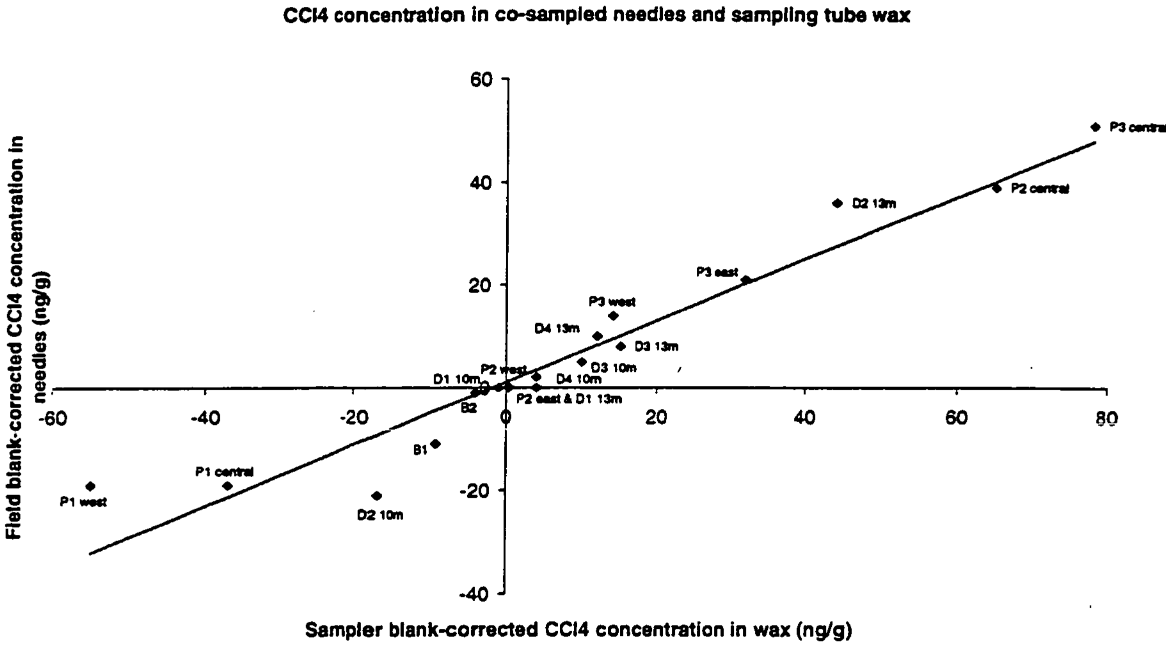


Figure 7.2.2 CCl_4 concentration in co-sampled needles and sampling tube wax.

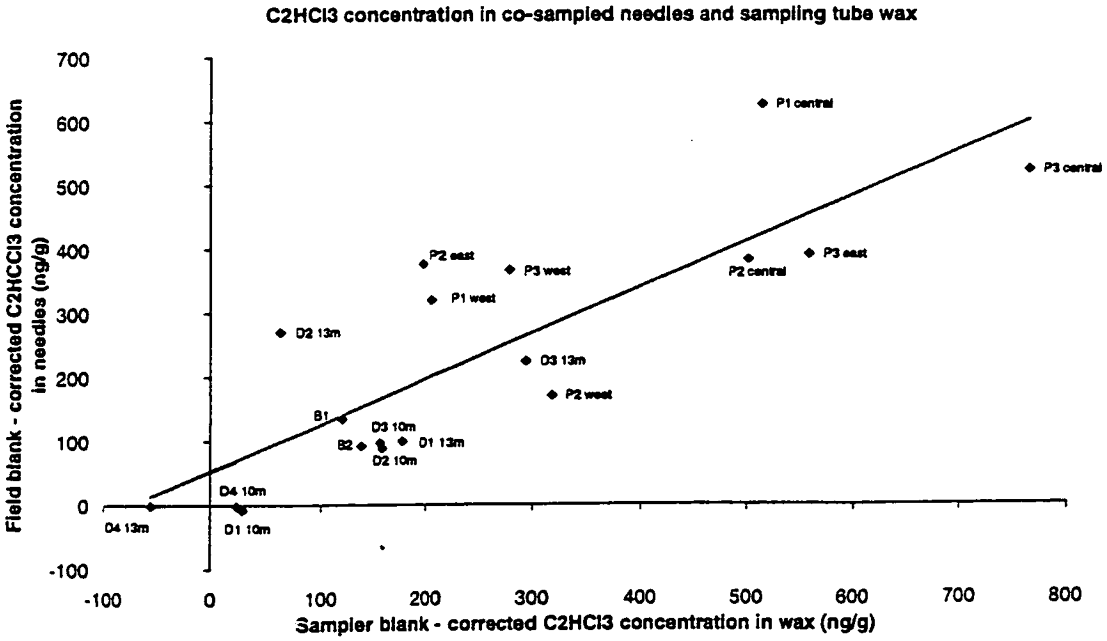


Figure 7.2.3 C_2HCl_3 concentration in co-sampled needles and sampling tube wax.

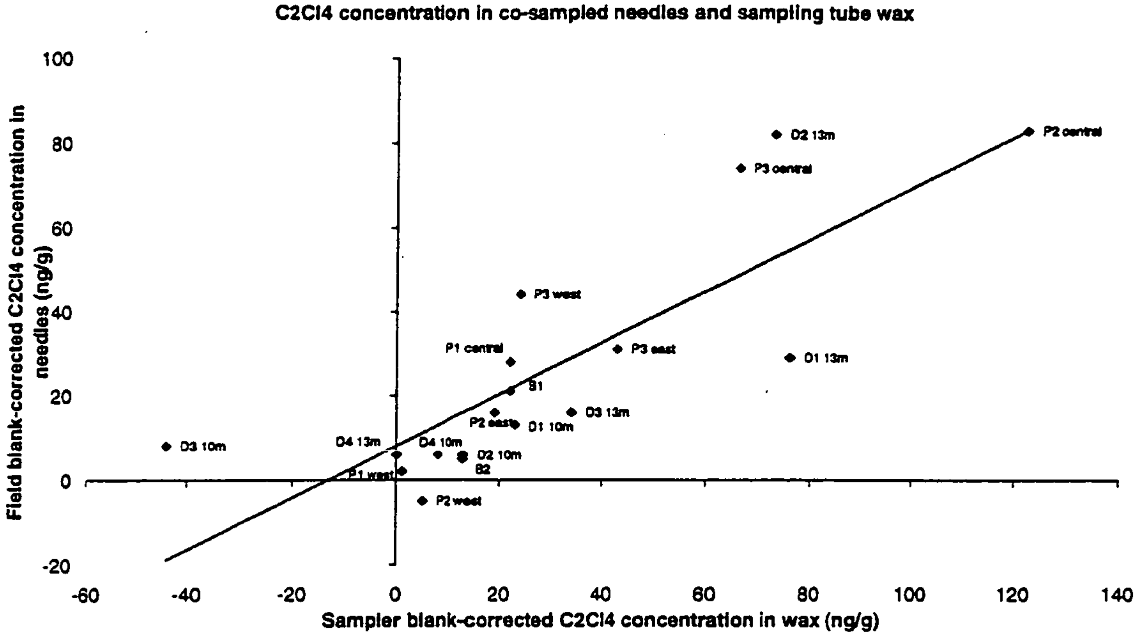


Figure 7.2.4 C_2Cl_4 concentration in co-sampled needles and sampling tube wax.

The ratio of the partition ratios determined between air and needles and air and wax is given by the slope of the ng/g$_{\text{dry weight}}$ Vs ng/g$_{\text{wax}}$ plot, i.e,

Ratio (of partition ratios) =

$$\frac{concentration\ in\ needles\ (g.g_{dryweight}{}^{-1})\ /\ concentration\ in\ air\ (gm^{-3})}{concentration\ in\ wax\ (g.g_{wax}{}^{-1})\ /\ concentration\ in\ air\ (gm^{-3})}$$

The laboratory-determined ratio of the partition ratios (dry weight basis) was plotted on the graph for comparison. Note that the constrained (through zero, negative points excluded) data is used in the final estimation of the similarity of fit to the laboratory-determined ratio of partition ratios shown in **Figure 7.2.5** and **Table 7.2.2**.

Table 7.2.1 Slope and intercept data from ng/g$_{\text{dry weight}}$ needle Vs ng/g wax linear regression plots.

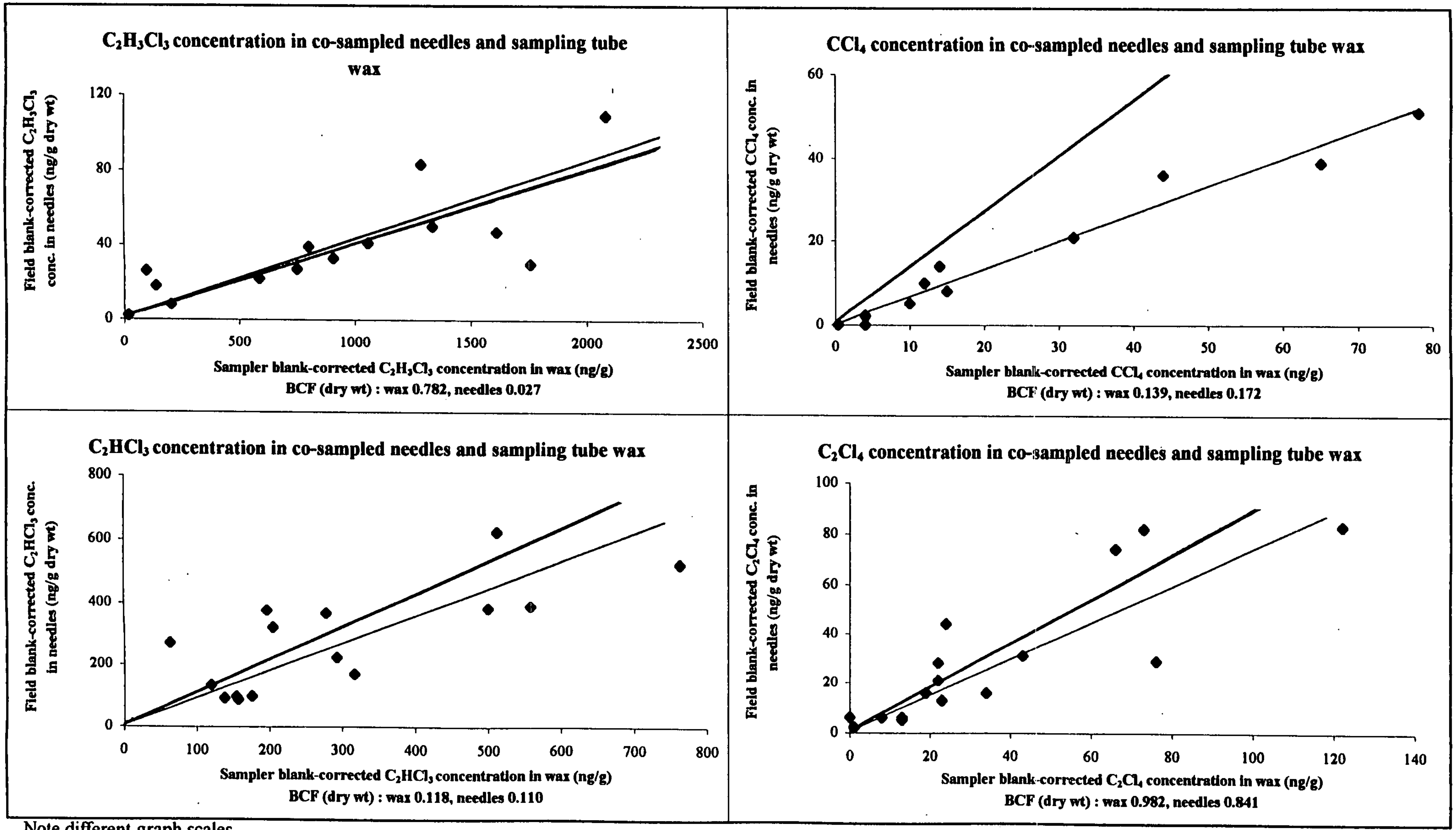
	Non-constrained, negative values included					Non-constrained, negative values not included					Constrained, negative values not included				
	slope	error on slope	intercept	error on intercept	r^2	slope	error on slope	intercept	error on intercept	r^2	slope	error on slope	intercept	error on intercept	r^2
$C_2H_3Cl_3$	0.037	0.006	2.6	5.7	0.70	0.034	0.008	7.7	8.5	0.58	0.04	0.004	0	N/A	0.51
CCl_4	0.6	0.043	1.1	1.4	0.92	0.67	0.04	-0.17	1.3	0.96	0.66	0.027	0	N/A	0.88
C_2HCl_3	0.72	0.12	54	40	0.66	0.63	0.15	90	54	0.53	0.85	0.09	0	N/A	0.40
C_2Cl_4	0.61	0.11	8.1	4.9	0.65	0.71	0.12	4	5.5	0.71	0.77	0.077	0	N/A	0.66

Errors are standard regression error for slope and intercept respectively

Table 7.2.2 Similarity of fit between air sampler wax partition ratio data and laboratory-determined data. Errors given are the standard error (se) of the slope. The 95% confidence levels were calculated as (2 x √n x se), (Miller and Miller, 1994). The needle partition ratios expressed as (g$_{\text{fresh weight}}$.m^{-3}/gm$^{-3}_{\text{air}}$) basis for comparison with other authors (Chapter 4) were converted to (g.g$^{-1}_{\text{dry weight}}$ /g.g$^{-1}_{\text{air}}$) using the average fresh weight/dry weight ratio (Appendices 6 and 8).

Chlorinated solvent	**Ratio of partition ratios determined using air sampler wax and needle (dry weight) data**	**Ratio of partition ratios determined using laboratory data on a fresh weight basis (Chapter 4)**	**Do 95% confidence intervals overlap?**	**Ratio of partition ratios determined using laboratory data expressed on a dry weight basis**	**Do 95% confidence intervals overlap?**
$C_2H_3Cl_3$	0.040±0.004	0.025±0.002	Yes	0.035±0.002	Yes
CCl_4	0.66±0.03	0.88±0.06	Yes	1.2±0.09	Yes
C_2HCl_3	0.85±0.09	0.65±0.08	Yes	0.93±0.12	Yes
C_2Cl_4	0.77±0.08	0.59±0.09	Yes	0.86±0.16	Yes

Figure 7.2.5 Concentration in co-sampled needles and sampling tube wax showing laboratory-determined partition ratio (as BCF on a dry weight basis) for comparison



Note different graph scales
Regression line shown as light line on graph
Partition ratio shown as bold line on graph

The geometric means from co-sampled and non-cosampled wax data were compared using the population t-test, and also with the needle data across all time. The results from the t-tests used to compare $\log_e$ geometric mean concentrations of co-sampled and other wax data for each compound are given in **Table 7.2.3(a,b)**. As the underlying population of concentration over time is sampled twice, once at the same time as the foliage and once at other times, the question must be asked whether these independent predictions are good estimates of the underlying population. No significant differences between the sample means were observed either over time or between sites. The wax data can therefore be combined to give a total population across all forests and all time (Figure 6.1.1). The needle data for the two year classes were also combined as is indicated by the ANOVA tests (Tables 6.4.1.2-4). The two data sets for needles were also not significantly different at the 95% level indicating that the two independent sets of samples may be good estimates of the underlying population over time.

Table 7.2.3 Results from t-tests used to compare $\log_e$ geometric mean concentrations (ng/g dry weight) for co-sampled and other data in (**a**) air sampler wax and (**b**) needles. Note that the needle data is combined year class (1992 and 1993) over the year. Note that **Figure 6.1.1** shows the needle classes separately at the three sites. Results which are significantly different at the 95% level are in **bold**. For some data sets, n was too small, or there were too many negative values, to calculate the $\log_e$ geometric mean and standard deviation.

(**a**) Log_e geometric mean concentrations (ng/g) in wax for co-sampled and non co-sampled (other) air sampler tubes, and the combined data set.

Compound	Forest	Co-sampled wax data	Other wax data	Population t-test on $\log_e$ geometric mean values (t statistic)	Log_e geometric mean concentration of combined data
$C_2H_3Cl_3$	Banchory	*	*	N/A	5.1±1.0
	Ponsonby	6.5±0.9	*	N/A	5.9±1.2
	Devilla	6.0±0.4	4.9±1.7	1.9	5.7±1.4
CCl_4	Banchory	*	2.6±0.9	N/A	1.9±1.3
	Ponsonby	2.9±1.5	*	N/A	1.8±0.8
	Devilla	2.1±1.3	2.6±0.9	0.9	2.4±0.9
C_2HCl_3	Banchory	*	4.2±1.6	N/A	4.5±0.8
	Ponsonby	6.0±1.6	4.5±0.5	2.1	5.2±1.0
	Devilla	4.7±0.9	4.9±1.3	0.4	4.8±1.0
C_2Cl_4	Banchory	*	2.6±0.5	N/A	2.7±0.4
	Ponsonby	3.6±1.9	1.7±1.3	2.1	2.6±1.5
	Devilla	3.1±1.3	3.2±2.0	0.1	3.1±1.0

* insufficient data to establish population mean

(b) Log$_e$ geometric mean concentrations (ng/g dry weight) in needles showing co-sampled, other and combined data.

Compound	Forest	Co-sampled needle data	Other needle data	Population t-test on log$_e$ geometric mean values (t statistic)	Log$_e$ geometric mean concentration of combined data
$C_2H_3Cl_3$	**Banchory**	*	2.8±0.7	N/A	3.2±0.4
	Ponsonby	3.3±1.3	*	N/A	2.9±1.4
	Devilla	3.5±0.4	2.5±2.6	0.3	3.0±1.2
CCl_4	**Banchory**	*	*	N/A	0.1±1.0
	Ponsonby	1.7±1.2	2.0±0.2	0.5	2.0±1.4
	Devilla	1.5±1.7	1.4±0.8	0.1	1.4±1.0
C_2HCl_3	**Banchory**	*	4.7±1.3	N/A	4.7±0.7
	Ponsonby	6.0±0.3	*	N/A	5.8±0.4
	Devilla	4.6±0.9	5.4±0.7	1.8	5.0±0.9
C_2Cl_4	**Banchory**	*	4.1±1.0	N/A	3.6±0.8
	Ponsonby	3.6±1.8	1.8±1.6	1.6	2.8±1.4
	Devilla	2.4±1.6	2.7±0.9	0.5	2.6±1.7

* insufficient data to establish population mean

7.3 Discussion and comparison with previous studies

Figure 7.2.5 shows that the ratio of the partition ratios determined from the field measurements is similar to the ratio of the partition ratios (dry weight basis) measured in the laboratory (Chapter 4).

The wax sampler tubes control diffusion and therefore remove turbulence effects, but sample in a similar way to needles. Measurements from the passive air samplers can consequently be used to estimate the concentrations of chlorinated solvents in needles directly. The average concentrations of the surrounding air, determined by the wax sampler tubes, indicate the levels present in the needles, and as such offer an excellent method of monitoring needle chlorinated solvent concentrations. This is confirmed by the good agreement between the co-sampled and other wax and needle data (Table 7.2.3).

The needle data are very variable, and difficult to collect, but will be related on average to air concentrations in the preceeding few hours. The time required for an equilibrium to be established between the atmosphere and the wax or needles (t_e) at high atmospheric concentrations generated in the laboratory is 2-6 hours (Chapter 4). The calculated equilibrium times for the wax in the diffusion tubes at the lower concentrations of ambient air are longer. These are given in **Table 7.3.1** (refer to Appendix 1 for equations).

The results in **Table 7.3.1** suggest that concentrations in the wax will only be related to the wind speed/direction of the previous 24 hours (at most). It is, however, still useful to consider the general wind patterns of the week preceeding sampling at each forest (as in Chapter 6).

Table 7.3.1 Times for equilibrium to be established between ambient air and wax in passive sampler tubes. Wax : air partition ratios (fresh weight basis) are given in Chapter 4, diffusion coefficients are taken from Underhill and Feigley (1991).

Chlorinated solvent	**Diffusion coefficient (D, m^2/s)**	**Wax : air partition ratio (*K*, $g.m^{-3}/g.m^{-3}$)**	**†Calculated equilibrium time (t_e, hours)**
$C_2H_3Cl_3$	8.55×10^{-6}	530	11.8
CCl_4	8.57×10^{-6}	100	2.2
C_2HCl_3	8.74×10^{-6}	80	1.8
C_2Cl_4	7.86×10^{-6}	680	16.6

† note that these are the calculated minimum values of t_e, the calculation in Appendix 1 assumes rapid diffusion in the wax layer, but this may in fact be 'slow'.

If, however, air concentrations are calculated from the wax data directly using these wax:air partition coefficients, unrealistically high concentrations are predicted. For example;

Wax contains up to 80ng/g CCl_4 @ 0.82g/cm^3
$\Rightarrow$ 66 ng/cm^3 or $0.66 \times 10^5 \mu g/m^3$
Partition ratio for wax is 100,
$\Rightarrow$ air concentration of 660μg/m^3 i.e. approximately 100 x too large.

This suggests that the phase equilibrium is non-linear. A non-linear phase equilibrium is also suggested by the surprisingly large ratios of partition ratios (except $C_2H_3Cl_3$) (Table 7.2.2). If most of the solvent is in the wax, and wax is approximately 1% dry weight, the ratio of ratios would also be expected to be approximately 1%. If the results are interpreted as the attainment of a non-linear equilibrium (wax : air) in the wax, it may not be necessary to invoke biphasic behaviour in the needles (needle : air) to interpret the sorption data for whole needles, as suggested by some authors.

One such example of possible biphasic uptake of C_2Cl_4 by spruce needles (as a representative chlorinated solvent) is given by Frank and Frank (1989). They propose that one compartment, which is small and quickly saturated, is characterised by a high partition ratio of 520 ($g.m^{-3}/g.m^{-3}$) at low concentrations (**Figure 7.3.1**). The second, larger, compartment at higher concentrations was thought to represent much slower uptake with a lower partition ratio of 50 ($g.m^{-3}/g.m^{-3}$).

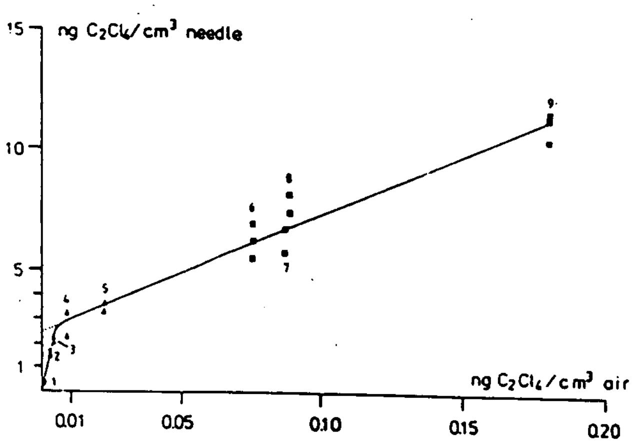


Figure 7.3.1 Correlation of atmospheric levels and the amounts extracted from spruce needles. (●) forests, (▲) city and (■) exposure chamber. Adapted from Frank and Frank, 1989.

Schreiber and Schönherr (1992) reported a dual-phase uptake for the high molecular weight chlorinated compounds pentachlorophenol and 2,4-dichlorophenoxy-acetic acid into conifer needles. They too suggest that the first rapid phase is attributable to sorption to the needle surface and the second slower phase represents penetration across cuticles and accumulation in the needle interior. They suggest that the first compartment is one in which reversible sorption occurs and from which the amounts taken up can be recovered by desorption. The relative contribution of the reversible fraction decreases with time. This fraction may itself be contained within two compartments - sorption to the exterior of the cuticular wax surface and sorption in the surface wax. They propose that irreversible binding to the leaf interior occurs as a result of cuticle asymmetry. Re-diffusion across the outer wax barrier is kinetically hindered, the diffusion coefficient through the outer 10% volume (wax-encrusted, crystalline) being several orders of magnitude less than that through the inner 90% volume (Schönherr and Riederer, 1989). This 90% volume forms a large sorption compartment for organic molecules but does not contribute much to the cuticle barrier properties. Molecules diffusing across the outer barrier into this inner volume will be rapidly desorbed by the apoplast and continue into the symplast. These phenols are, however, rather polar so we cannot deduce that chlorinated solvents behave in the same way.

A two compartment model has also been proposed for the uptake of gaseous DDE into spruce needles (Hauk *et al.*, 1994). They suggest that one compartment reacts within hours to changes in environmental concentration, the second compartment taking months to react.

The agreement between needles and wax from my study suggests that chlorinated solvents are not retained/accumulated, but are in fairly good equilibrium with the atmosphere. No evidence for the existence of an irreversible compartment at very low concentrations can be seen from either the wax extract partition ratio plots (Figure 4.3.3.3) or the data in Figure 7.2.5 (ratio of partition ratio plots) - the intercept, although on the needle side, not being statistically significant at the 95% level (Table 7.2.1).

A non-detectable intercept would be expected bearing in mind the high concentrations used for the partition ratio determination in this study (2.5-1500 mg/m^3). Frank and Frank (1989) obtained a value of only 2.5 ng/cm^3 (given by the intercept) for the capacity of the first, smaller compartment proposed in the biphasic uptake plot for fresh spruce needles **(Figure 7.3.1)** exposed to ambient (forest and city) concentrations of up to 20 µg/m^3 of air. An intercept of this magnitude would not be detectable at the high atmospheric test concentrations of this study, even assuming that the biphasic uptake mechanism operates. These results were confirmed by Figge (1988) working with spruce needles at air concentrations of 10-200 mg/m^3.

If more representative concentrations for determining the wax partition ratio (concentration in wax/concentration in air) had been used, the wax plot (Chapter 4) would have been expected to go through the origin. A significant intercept for the needle data would have suggested that an irreversibly bound fraction was present and more than one compartment existed (Table 4.3.3.2).

Confirmation of the presence, or correct assignment, of any compartments that may exist is not possible without analysing the desorption kinetics of chlorinated solvents from conifer needles (Schreiber and Schönherr, 1993) or further partition ratio work in conifer needles at very low concentrations. Neither the needle or wax concentration data collected from the three forests in this study can yet be used to estimate air concentrations.

In addition, all the data below the 5µg/m^3 'cut off' (on which the dual phase uptake concept is based) seen on the plot presented by Frank and Frank (1989, **Figure 7.3.1**) is from forest or city samples rather than exposure chamber studies. The problems associated with accurate and reproducible generation of standard atmospheres of chlorocarbons at such very low concentrations are complex (refer to Chapter 8). In addition, these forest and city samples were all collected at different ambient temperatures (recorded in original paper) and are therefore not directly comparable.

The partition ratio data published by Frank *et al.* appears to be contradictory. A partition ratio of 50 (g.m^{-3}/g.m^{-3}) for C_2Cl_4 between needles and air at the higher air concentrations (1989) is contradicted by a previously published work (Frank and Frank, 1986) which suggested that the corresponding ratio was 95 (g.m^{-3}/g.m^{-3}). Both are expressed on a fresh weight basis. It is noted that the wax partition ratio of 2200 g.m^{-3}/g.m^{-3} obtained in the laboratory using the total lipid/wax content of *Picea abies* needles extracted with cyclohexane and chloroform/methanol (1986) is very different from the 'wax compartment' of the biphasic model which has a wax:air partition ratio of 520 g.m^{-3}/g.m^{-3} (Frank and Frank, 1989). This ratio of 520 is in fact similar to the beeswax data cited in the 1986 paper (740 g.m^{-3}/g.m^{-3}). This similarity is to be expected as partitioning into the beeswax system, rather than into an extractant which also contains internal lipids, is more representative of uptake into the epicuticular wax. The equivalent partition ratio obtained for the pine wax in this study (700

$g.m^{-3}/g.m^{-3}$) is also similar to the beeswax result (see **Table 4.3.3.3** below - repeated from Chapter 4). It is suggested, therefore, that the Soxhlet procedure using hexane in this project is a less disruptive extraction technique than chloroform (Frank and Frank, 1986). Only the surface layer of wax from the needle is removed rather than much of the internal lipid also being stripped out (4.5% of the fresh weight extracted).

Table 4.3.3.3 (repeated) Partition ratios ($g.m^{-3}/g.m^{-3}$) of chlorinated solvents between lipid and air (21°C). Needle data on fresh weight basis.

Chlorinated solvent	Scots pine lipid/wax extract	Total wax & lipid of Spruce†	Beeswax†	Scots pine needles	Spruce needles†
$C_2H_3Cl_3$	500	-	-	13	-
CCl_4	100	400	140	84	17
C_2HCl_3	80	1000	270	53	44
C_2Cl_4	700	2200	740	399	95‡

†From Frank and Frank, 1986. Temperature 22°C. Total lipid/wax (4.5% of fresh weight) extracted with cyclohexane and chloroform/methanol (2:1, v/v).
‡Value of 129 from Figge, 1988.

It is important to recognise that the facilities for active sampling of air (thermodesorption, cryogenics, dedicated on-site GC and autosampler) were not available during this project. Direct determination of concentrations of airborne chlorinated solvents, by cartridge or direct sampling and thermodesorption, as used by other authors (Frank and Frank, 1988b; Sturges and Taylor, 1990) was not possible without this equipment. The passive air sampling technique used instead tackled the problem from another direction - via the partition ratios, and as such has been shown to act as a simple and cost-effective tool for prediction of needle data from concentrations determined in the wax adsorbent. This data is in many ways more useful, as the actual levels taken up by the needles are measured rather than the surrounding air concentration.

Chapter 8

Conclusions and discussion of relevance to forest decline

‘Begin at the beginning,’ the King said gravely,
‘and go on till you come to the end: then stop’

Lewis Carroll

8. CONCLUSIONS AND DISCUSSION OF RELEVANCE TO FOREST DECLINE

8.1 Summary conclusions

(1) An initial data base for chlorinated solvent concentrations in three forests in northern Britain has been established. The concentrations of the chlorocarbons $C_2H_3Cl_3$, CCl_4 and C_2Cl_4, found in Scots pine needles in a rural British forest and two forests near potential industrial sources are similar to those measured at equivalent locations in central Europe. The spread of results is however greater. Direct comparisons are not possible due to different expressions of concentrations on a fresh weight, dry weight or area basis and the use of different tree species. The average geometric mean concentrations in ng/g dry weight are: $C_2H_3Cl_3$ 20; CCl_4 5; C_2Cl_4 30. The concentrations found in Britain for C_2HCl_3 (typical mean: 130 ng/g dry weight) are greater than those reported for forests in rural and industrial/urban locations in central Europe. The concentrations recorded from the clean air site are lower but there is no statistically significant difference between the median concentrations seen at this forest and the two forests near industry over the 15 month sampling time.

(2) The chlorocarbon concentrations measured in the needles are proportional to amounts measured in passive air sampling tubes containing an extract of Scots pine wax and exposed to the atmosphere for one month. Air sampling tubes can therefore be used as a simple surrogates for predicting actual amounts in needles.

(3) No significant differences in the content of chlorinated solvents were found between the 1992 and 1993 year classes for *Pinus sylvestris* needles collected at any of the three forests, showing that these compounds do not accumulate in needles.

(4) The Sellafield factory appears to have no influence on chlorocarbon concentrations observed in the adjacent Ponsonby Tarn forest. No concentration gradient away from the site is seen; the more exposed central site concentrations are highest. The petrochemical industry based at Grangemouth may be linked to concentrations found in Devilla forest northeast of the industry across the river Forth estuary. A vertical concentration gradient (positive) through the canopy is seen, indicating the source to be airborne. Chlorocarbon emissions from Sellafield are thought to be considerably less than those from Grangemouth.

(5) The partitioning of chlorinated solvents between whole needles and surface wax can be derived from the ratio of the partition ratios determined in the field for (chlorocarbon conc^n in whole needles /conc^n in air) and (wax conc^n /air conc^n). Partitioning between needles and surface wax from field samples was similar to that found in samples from very much larger concentrations in the laboratory. This suggests that the phase equilibrium between pine needles and air is non-linear with air concentration. The unrealistically large air concentrations predicted from field exposed wax data using the laboratory-determined wax:air partition coefficient also suggest non-linearity at ambient field concentrations. Neither the needle nor wax concentration data collected from the three forests can yet be used to estimate air concentrations. The partition ratio at low concentrations ($<5\mu g/m^3$) may be required for conversion of air sampler wax data into absolute air concentrations.

8.2 General discussion

In this study, the geometric standard deviation of chlorocarbon concentration in pine needles generally falls between 2.0 and 3.0 (Table 6.1.4). These values are similar to those found for air pollutants by other workers (Fowler and Cape, 1982), and suggest that the atmosphere is the primary source of the compounds found in the needles. The chlorinated hydrocarbon concentrations can therefore be correlated with wind speed and direction, the log normal distribution of pollutant concentration (demonstrated in Figure 5.7.1) being shown to relate to the same forms of wind speed (Fowler and Cape, 1982; Mage, 1980).

8.2.1 Epicuticular waxes

As epicuticular waxes are one of the first targets of air pollutants on plants (as with all atmospheric influences), they can be used as an early indicator of air pollution effects (Hellqvist *et al.*, 1992; Turunen and Huttunen, 1990). However, as the epicuticular wax structures are not exclusively affected by air pollutants, but also by other artificial and natural agents, assignment of specific influences to particular damage is difficult (Bermadinger-Stabentheiner, 1993).

The structure and quantity of wax depends on internal factors such as needle age and external factors such as geographical situation (Guenthardt, 1984). It has been reported that the variation in natural structural wax degradation can overshadow possible effects from air pollution (Günthardt-Goerg *et al.*, 1994). Wax tubes formed in spring on new needles fuse and erode during the course of the year as part of the natural ageing process. This structural degradation (effected primarily by wind and precipitation) depends on the exposure of the wax within the needle, branch, tree, stand and site and most probably has no phytopathological value, with little link to air quality (Günthardt-Goerg *et al.*, 1994).

The different structure and composition of epicuticular wax may explain the apparent species selectivity of the tree decline. The most heavily damaged species appear to have well- developed coherent epicuticular wax layers which increase with exposure to light (Frank and Frank, 1986; Napp-Zinn, 1966). Needle life and needle thickness increase with altitude as does quantity of epicuticular wax and the dry weight per needle (Günthardt-Goerg , 1986).

The quantitative differences in wax composition are thought to change with different geographical situation, particularly for *Picea abies* which is known for its genetic variability (Günthardt-Goerg, 1986). The secondary alcohol nonacosan-10-ol is usually a main component in conifers, *Pinus sylvestris* also has a large quantity of free fatty acids and primary alcohols.

Quantitative differences in relative amounts of the wax components develop over time, the changes in the wax structure being marked for species such as *Picea abies* and *Pinus cembra,* with amounts of nonacosan-10-ol and free fatty acids increasing with needle age and free ω-hydroxy fatty acids and the secondary alchohol hentriacontan-4-ol decreasing. The main quantitative increase and chemical development of epicuticular wax is during the first vegetation period. Changes in epicuticular wax composition after a leaf or needle is fully expanded are generally thought not to occur. The amount of wax has been shown to decrease with increasing age (Günthardt-Goerg, 1986).

It is therefore not surprising that the concentrations observed in the pine needles are different, and generally greater, than those seen in spruce. It is also shown that the leaf waxes of different species may display significant differences in their ability to accumulate these chlorocarbons, or even that the variation between individual trees might be large. Investigations at different forest sites suggest that levels of chlorocarbons in conifer needles change in dependence on the age of the needles as well as on the regions (Plümacher and Renner, 1993), a trend documented for other higher molecular weight chlorinated hydrocarbons (Gaggi and Bacci, 1985). However, the evidence from the measurements made in my study indicates that the representative compounds of this group of volatile chlorocarbons are not accumulated in the needles.

No significant concentration differences between the 1992 and 1993 year class needles were found (Tables 6.4.1.2-4). This is in agreement with findings that there is no increase, and possibly even a decrease, in the amount of wax per unit area over the lifetime of needles (Günthardt-Goerg, 1986). This would suggest that while it is recognised that the phytotoxic effects of these compounds are important, the direct threat to forest health from this group of chemicals may well be less than previously thought (Frank, 1991). The effects of their degradation products may well be of more significance (Section 8.2.3).

8.2.2 Forest decline

The extent of forest decline observed in the UK is much less than that of central European forests (Cape *et al.*, 1988; Department of the Environment report, 1993; Innes, 1991; Mather, 1994). The existence of similar concentrations of chlorocarbons in the British forests to those found on the continent strongly suggests that these air pollutants are not major causative agents of the widespread chlorosis and defoliation observed in central Europe. However, even if the exact mechanisms are unknown, it may well be important to measure observed surface properties and concentrations of chemicals in the needles of apparently healthy trees so that those at risk of developing the symptoms of 'decline' can be identified (Cape *et al.*, 1993). Controlled exposure of trees to a pollutant, or pollutant mixture, is necessary before any statement regarding casual links between air pollutant concentration and observed changes in the field can be made. It has been suggested that the wax tubes which characterise the form of wax around the stomata in Scots pine are associated with ketones and secondary alcohols (Baker, 1982). Any decrease in the amounts of these components resulting from the effects of any environmental influence, including air pollutants, could affect the gas exchange characteristics of the needles and consequently the uptake and enrichment of chemicals. There is, however, no evidence of a specific chemical effect which would identify the causal agent(s) (Cape, 1986).

The presence of this group of compounds in the air may, however, contribute to micromorphological injury to the epicuticular waxes such as degradation from the crystalline (tube) to amorphous state (Sauter and Pambor, 1989; Turunen and Huttunen, 1990). This is serious as the epicuticular wax is the outermost coating of the needles, and its quality, structure and composition are essential for tree survival (Guenthardt, 1984).

The erosion of epicuticular waxes corresponds well to air pollutants levels and may well be a relevant factor of the multiple forest decline syndrome (Hellqvist *et al.*, 1992). The needles from trees at polluted sites have been shown to have less wax than clean air sites (Cape, 1986), several components (secondary and primary alcohols) of the wax decreasing in amount over time. The rate of change is greater in polluted air. Erosion of waxes can alter needle wettability (Boyce and Berlyn, 1988) as measured by contact angles for water droplets (Cape, 1986), and rain retention. This can result in enhanced leaching of nutrients and uptake of pollutants as permeability of the cuticle increases. The gas exchange of the needles is regulated by the wax layer and transpiration rates are altered as stomatal and resistance to diffusion rates are increased or decreased. The conifers are predisposed to various environmental stresses - both biotic and abiotic - particularly by exposure to precipitation and abrasion by wind (van Gardingen *et al.*, 1991).

8.2.3 Chlorocarbon degradation products

It has been suggested that the high concentrations of the chlorocarbon degradation product trichloroacetic acid (TCA) found in needles (up to : 175 ng/g fresh weight pine needles, Plümacher and Renner, 1993; 126ng/g fresh weight spruce needles, Frank and Frank, 1992) are also linked to the forest decline in Scots pine (Frank *et al.*, 1994) and Norway spruce (Frank *et al.*, 1992).

The assumed pathways for TCA formation are metabolism of chlorocarbons inside the needles (air path) or atmospheric photo-oxidation and wash-out by rain of this water-soluble reaction product, resulting in accumulation in soil, and subsequent uptake with the transpiration flow (refer to Chapter 1). There is disagreement regarding the atmospheric oxidation step as the degraded chlorinated pollutants would mainly end up as phosgene and chlorine (Hoekstra and De Leer, 1995), which would not explain the large TCA concentrations seen. There is also some evidence for the production of TCA in soil (Schroll *et al.*, 1994). The TCA concentrations found in needles of different species of tree may, however, reflect those of chlorocarbons. For the data presented by Plümacher and Renner in 1993 for C_2Cl_4 (pine :8.3 ng/g fresh weight, spruce 4.6 ng/g fresh weight, same forest and time) and TCA (up to 175 ng/g fresh weight in pine, same forest) and Frank *et al.* in 1992 (TCA in spruce, up to 126ng/g fresh weight, different forest), it can be seen that greater amounts of both compound are found in pine rather than spruce. Care must be taken when comparing data from different forests measured at different times.

The phytotoxic and herbicidal properties of TCA (and monochloroacetate(MCA), also found at high levels in needles; Frank *et al.*, 1994) are well documented (Morrison and Cohen, 1980). TCA is known to cause chlorosis, inhibition of growth and disturbance of cuticle development in sublethal quantities. It is accepted that TCA and other haloacetic acids should also be considered when evaluating the effects of volatile chlorocarbons on forests and their contribution to present forest decline (Frank, 1991; Frank *et al.*, 1994; Plümacher and Renner, 1993 *in press*).

8.2.4 Potential future studies

Some suggestions for future work are presented in this final section. The interest in the phenomenon of forest decline in so-called clean air regions in Europe has stimulated much research. However, very little work in this context in Britain has been done on this group of volatile chlorinated hydrocarbons. There are many ideas to pursue, and much scope for imaginative follow up of this and other reported work.

(i) Haloacetates

The possible link between haloacetates, as degradation products of chlorinated solvents, and forest decline has been discussed. Measurement of TCA and other haloacetic acids in the foliage, air and soil of British forests is required for comparative purposes with central European data. A preliminary study carried out in 1994 at the University of Edinburgh in conjunction with ITE Bush suggested that TCA levels in Sitka spruce (*Picea sitchensis*) needles at Glentrees forest, near Peebles, Scotland, (no obvious source of pollution) were similar to those found in central Europe (Vaughan, 1994 unpublished data). A headspace method using decarboxylation of TCA to chloroform was used with GC-ECD analysis (Plümacher and Renner, 1993 *in press*). Results obtained from these preliminary studies at this forest were:

1992 needles	150m a.s.l.	300±10 ng/g dry weight
1993 needles	150m a.s.l.	280±80 ng/g dry weight
1993 needles	450m a.s.l.	1300±800 ng/g dry weight

The vertical gradient is marked and suggests that wet input is the dominant uptake pathway, cloud capture and rain input increasing with altitude (Miller *et al.*, 1993). Gas phase input would show a more uniform spread of results due to the mixing of the air mass by winds.

This TCA study should be extended to include more spruce needle year classes, to see if there is more accumulation in older needles, and also different species of tree. Study of Scots pine needles from the three forests of my study would allow correlation of TCA concentrations with known chlorocarbon data. Other haloacetates could be looked for (Frank *et al.*, 1994) and the vertical profile through the canopy considered.

(2) Exposure chambers

Controlled environment chambers have previously been used to investigate the effects of environmental influences on development of spruce needles cuticles (Cape and Percy, 1993). Some possible studies into the effects of chlorocarbon (and TCA) concentrations at known standard atmospheres on needles of different age classes are described below. Exposure of needles to individual or mixtures of compounds would demonstrate whether one or more air pollutants could produce similar changes to those observed in the field (Cape *et al.*, 1993 *in press*). It is possible that diagnostic tests for chlorocarbons/TCA could be evaluated which would aid in the interpretation of measurements of properties made on trees in forests. Air sampler tubes as described in this study could be used in parallel.

The generation of reproducible low concentrations of atmospheric vapours may be achieved with permeation tubes as used for calibration standard preparation for sulphur-containing compounds (Carroll, 1985). The permeation tubes are used to generate a very small, precisely known flow of compound (Kin-tek laboratories, Inc). This permeate flow is mixed with a much larger flow of dilutent gas (N_2) to form trace gas concentration mixtures (sub ppb). Gas standards can be blended in the laboratory in precisely known proportions (491M Modular Gas Standards Generator), bottled (or piped) and released as a controlled environment into the chamber at a known rate. The use of this technique may well be limited by cost.

Experiments could be designed around the following ideas:

(i) Young trees in exposure chambers over winter in an atmosphere of high solvent concentration (single and mixtures of compounds) may exhibit phytotoxicological damage when exposed to the high ultraviolet light levels of the following spring. Comparisons of uptake rates and effects should be made with control trees grown in clean exposure chamber conditions.

(ii) Measurement of the partitioning characteristics of needles grown on trees exposed to (known) very low atmospheric concentrations. Such data could provide evidence for a possible biphasic uptake, as proposed by Frank and Frank (1986) working with spruce, in Scots pine, and other tree species. The laboratory partition ratio studies for the needles and wax extract performed at high air concentrations could also be repeated using very low concentrations.

(iii) The haloacetate input and output pathways could be studied during different growth phases of the tree. The tree is exposed to combinations of known atmospheric and/or soil concentrations of labelled TCA/MCA etc. and the recovery from the needles determined using radioactive assay.

(3) SEM techniques

SEM is a powerful technique which has been used by many groups to detect changes in wax morphology or distribution in the context of air pollution studies (Günthardt-Goerg, 1986; Percy *et al.*, 1993 in press). It would be useful to support the exposure chamber work described above with SEM assessments of the extent of damage to epicuticular wax structure and composition.

(4) GC Methods

(i) The headspace technique used by Plümacher and Renner (1993 *in press*) for chlorinated solvent (and TCA) concentration determination in rainwater and pine needles merits investigation. A modified standard addition method for calibration with needles is used.

(ii) Preliminary trials using direct thermal desorption and an 'Optic' injector (Ai Cambridge Ltd) showed potential (Appendix 12). The headspace of a vial containing needles or exposed wax, either dry or extracted with solvent, is swept and trapped in a tube filled with an optimised adsorbent (off-line). This tube is transferred to the

'Optic' injector fitted in the GC where it is thermally desorbed directly onto the capillary column (Short Path Thermal Desorption). The transfer of the external sample is quantitative. The tube is essentially an injector liner packed with a suitable adsorbent. Two tubes in series could be used to determine breakthrough characteristics (Frank and Frank, 1988a). The results should be better than the equivalent purge and trap method as less extraction solvent is taken up by the adsorbent. The trials were not calibrated so measurements were not quantitative. Amounts of all five chlorocarbons were clearly seen in the needle sample, with no contamination problems for $CHCl_3$. Detection limits may be lower.

(iii) GC/MSMS

Further time is required for optimisation of the GC/MSMS technique and lowering of detection limits. The results from this work displayed its potential as a quantitative confirmational tool.

8.3 Closing remarks

The results from this study clearly demonstrate that the issue of chlorinated solvents in forested ecosystems merits further research, particularly in Britain, and that there are many interlinked strands that are worth investigating.

Of particular note, bearing in mind the concentration range common in so-called 'clean' air regions, is the possible link between concentrations of such compounds and their degradation products, and stress in trees.

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Appendix 1 : Passive air sampler theory

Principle of operation

The passive sampling technique is a practical application of Fick's first law of diffusion regarding gaseous species transfer through a fluid (Palmes *et al.*, 1976). Fick's law states that the mass transfer rate of gas during diffusion is directly proportional to the diffusivity of the gas in the fluid, i.e. air, the concentration gradient and the diffusion path cross-sectional area. The following expression can be written (Cao and Hewitt, 1991) :

$$J = -DA\frac{dC}{dX} \qquad (1)$$

where

J = diffusion transfer rate (g/s)
D = diffusion coefficient of the species in air (m^2/s)
A = effective cross-sectional area (m^2)
X = distance along the diffusion path (m)
C = analyte concentration at distance X (g/m^3)

The left hand side of the equation is negative because the analyte concentration decreases in the direction of diffusion.

If the concentration gradient (driving force for diffusion down the tube) is (C_1-C_0) over the total diffusional pathway (X_1-X_0 = L), equation (1) can be integrated:

$$\Rightarrow \qquad J = R\frac{DA(C_1 - C_0)}{L} \qquad (2)$$

and

$$Q = R\frac{DA(C_1 - C_0)t}{L} \qquad (3)$$

where

Q = mass uptake (g) [amount of species which passes through a section A (m^2) during time t (sec)]
L = length of the diffusional path (m)
R = as transfer resistance term (≤ 1, Bertoni *et al.*, 1990)
C_1 = external analyte concentration (g/m^3)
C_0 = analyte concentration at the interface of the adsorbent (g/m^3)
t = sampling time (sec)

If it is assumed that the adsorbent used is 100% efficient, the analyte concentration at the interface of the adsorbent (C_0) is assumed to be zero and the concentration gradient is equal to the ambient concentration of test gas. We can write:

$$Q = R\frac{DAC_1t}{L} \qquad (4)$$

However, this assumption is never true in practice, particularly for polymeric adsorbents with non-linear isotherms. If the optimum adsorbent for the analyte of interest is used, though, the error resulting from this approximation will be small and can be ignored when determining low levels of compounds in indoor and outdoor air.

The above expressions can be applied to all types of passive sampler, whatever type of barrier to diffusion is in place, e.g. air gap, membrane, air gap + membrane etc. The value of R in equations (2) - (4) will vary. When the diffusional path is 'open' (air gap) $R = 1$; when a medium such as a filter, septum or membrane is placed in the diffusion path, $R<1$.

Determination of Uptake Rate

The expression KDA/L (equation 4) is defined as the diffusive uptake rate. It has the same unit (cm^3/s) as conventional pumped sampler air flow rate and can be determined experimentally for any type of passive sampler (equation 5).

$$\textbf{Experimental Uptake Rate} \; (\text{ng.ppm}^{-1}\text{min}^{-1}) = \frac{\text{mass uptake (ng)}}{\text{analyte concentration (ppm) x exposure time (min)}} \qquad (5)$$

The **Ideal Uptake Rate** is given by the expression RDA/L on condition that K is known. For a given sampler, the value of A/L is constant. The diffusion coefficient (D) of a given species in air can be found in the literature (Lugg, 1968; Underhill and Feigley, 1991) or calculated (Pannwitz, 1984). When the diffusion barrier is an air gap and $R = 1$, the uptake rate = DA/L.

Equating the ideal and experimental uptake rates (equation 6) will allow C_1, the environmental air concentration, to be determined. The amount, Q, collected on the adsorbent is determined analytically and the exposure time (t) is known.

$$C_1 = \frac{QL}{RAtD} \qquad (6)$$

The diffusive uptake rate is only constant if C_0 remains equal to zero during the entire exposure time. When the sampled amount of the species begins to saturate the adsorbing surface and amounts are no longer negligible, C_0 increases, leading to a non-linear correlation between exposure time and amount sampled (Bertoni *et al.*, 1990).

The effective, rather than the total, cross-sectional area is used when calculating the ideal uptake rate, particularly for passive samplers, where the solid adsorbent is retained inside the sampling device by some barrier, e.g. a stainless steel gauze, which alters the total cross-sectional area.

Calculation of equilibrium time (t_e)

If it is assumed that the amount of solvent in the wax is Q(g)/V(volume of wax, m^3), the analyte concentration (C, g/m^3) can be defined as

$$C = \left[\frac{Q}{V}\right].K^{-1} \tag{7}$$

where K is the partition coefficient between wax and air (g.m$^{-3}{}_{\text{wax}}$/g.m$^{-3}{}_{\text{air}}$).
When $R = 1$ (air gap), the flux of material along the diffusion tube (g/s) is

$$J = \frac{dq}{dt} = \frac{DA}{L}(C_1 - C_o) = \frac{DA}{L}\left(C_1 - \left[\frac{q}{KV}\right]\right) \quad \text{(see equation 2)} \tag{8}$$

As the solvent accumulates in the wax, the 'surface' concentration rises and the flux decreases.

Let $\quad Y = C_1 - \left[\frac{q}{KV}\right],$

$$dY = -\left[\frac{dq}{KV}\right] \quad \Rightarrow \quad dq = -(KVdY)$$

rewriting the equation :

$$-KV\frac{dY}{dt} = Y\frac{DA}{L}$$

$$\Rightarrow \quad \frac{dY}{Y} = -\left[\frac{DA}{LKV}\right]dt$$

integrating:

$$\ln Y = -\left[\frac{DA}{LKV}\right]t + \text{constant}$$

or

$$Y = C'\mathrm{e}^{-\left[\frac{DA}{LKV}t\right]} \qquad \text{where } C' \text{ is the integration constant}$$

$$\Rightarrow \quad \left[C_1 - \left[\frac{q}{KV}\right]\right] = C'\mathrm{e}^{-\left[\frac{DA}{LKV}t\right]}$$

At $t = 0$, $q = 0$ therefore $C_l = C'$ and

$$C_1 - \left[\frac{q}{KV}\right] = C_1 e^{-\left[\frac{DA}{LKV}t\right]}$$

$$\therefore \frac{q(t)}{KV} = C_1(1 - e^{-\left[\frac{DA}{LKV}t\right]}) \text{ and } q(t) = KVC_1(1 - e^{-\left[\frac{DA}{LKV}t\right]}) \qquad (9)$$

When the wax is 99% saturated;

$$0.99 = 1 - e^{-\left[\frac{DA}{LKV}t\right]}$$

$$\Rightarrow e^{-\left[\frac{DA}{LKV}t\right]} = 0.01$$

$$\Rightarrow \frac{DA}{LKV}t = 4.61$$

$$\Rightarrow t = 4.61\frac{LKV}{DA} \qquad (10)$$

The time (t_e) for a mass of solvent (99% saturation) to attain equilibrium with C_l can be calculated from this equation. If all the wax is on the bottom of the tube, $V=A.h$ (h is depth of wax in m) and the equation simplifies to :

$$q(t) = q_\infty(1 - e^{-\left[\frac{D}{LKh}t\right]}) \quad \Rightarrow \quad t_e = 4.61\frac{LKh}{D} \qquad (11)$$

For example, the diffusion coefficient for $C_2H_3Cl_3$ is $8.55\text{x}10^{-6}\text{m}^2/\text{s}$ (Underhill and Feigley, 1991), K is 530 (Chapter 4), the length of the tube is 0.075m and the height of wax in the tube is 0.002m (Chapter 2) :

$$\frac{LKh}{D} = \frac{0.075\text{x}530\text{x}0.002}{8.55\text{x}10^{-6}} = 9298 \qquad \therefore 4.61 \text{ x } 9298 = t_e = 11.9 \text{ hours}$$

(for 90% saturation, t_e = 6 hours)

This calculation assumes that the diffusion in the wax is faster than the rate of equilibration with the air, which is unlikely to be true in practice as diffusion into and out of the wax will take longer (i.e. equilibration to a rise or fall in C_l). This is acceptable given the relatively thin layer of wax, and as the rate of solvent diffusion in the wax is not known.

Temperature and pressure effects

The mass uptake (Q) is a function of concentration (C) and the diffusion coefficient, D (equation 3).

i.e. $$Q = f(C, D) \tag{12}$$

The general equation for a gas also defines C;

$$C = \frac{n}{V} = \frac{p}{RT} \tag{13}$$

and D is a function of absolute temperature and pressure

$$d = f\frac{(T^{3/2})}{p} \tag{14}$$

combining (7, 8 and 9)

$$Q = f\left[\frac{p}{T}, \frac{T^{3/2}}{p}\right] \tag{15}$$

or $$Q = f(T^{1/2}) \tag{16}$$

The mass uptake of a passive sampler is therefore only affected by temperature and not pressure (Palmes *et al.*, 1976). The minor fluctuations of indoor temperatures will ensure the temperature effect is negligible for indoor air sampling. The broader range of ambient air temperatures may have some effect on passive samplers. This is only true when the sampling efficiency of the adsorbent is not temperature dependent. Temperature effects are therefore negligible and no correction is normally needed.

Face velocity, or ambient wind speed, effects

A minimum face velocity parallel to the open face of passive samplers is needed to ensure there is sufficient external flow over the sampling surface. The high uptake rate of badge-type samplers (high area : length ratio) demands a minimum face velocity of 0.05 - 0.1 m/s. This high face velocity can result in turbulence within the diffusion air gap. This effect is particularly important for ambient air sampling as wind velocities are usually high. Tube-type samplers are characterised by a small sampling surface. The influence of air speed decreases with a reduction in diameter (Bertoni *et al.*, 1990) and the minimum face velocity of tube-type samplers is 0.001m/s. A length : diameter ratio of greater than 2.5 - 3.0 (Harper and Purnell, 1987) will minimise external flow effects, as will the employment of draught shields.

Selection of the optimum adsorbent

The importance of selecting an optimal adsorbent for a specific analyte to maintain near-zero analyte concentrations at the adsorbent surface has been stressed (Section 2.2.1). This ensures that experimental uptake rates are as near constant as possible in accordance with ideal behaviour so long as adsorbent saturation does not occur. This reflects the dependence in the ideal case only on A, L and D.

Given the above calculations, it can be seen that in this case wax is not an optimal adsorbent for integrating concentrations over long periods, as saturation will occur over a day or less (Chapter 7). The solvent concentration in wax therefore reflects air concentrations during the day immediately preceeding sampling. The rate of equilibration of whole needles is likely to be faster, depending on wind speed, and probably only a few hours (Chapter 4).

Appendix 2 : Wax density calculations

The density of Scots pine wax was calculated as the mean value ± standard deviation of 11 individual measurements. The diameter of the capillary tube = 0.094cm. Measurements of length were made using a digital micrometer. Example calculations use data from capillary tube 1.

Capillary tube	Wax weight (g x10^{-3}), m	'Wax slug' length (cm), h	Wax volume (cm^3 x10^{-3}), v	wax density (g/cm^3), ρ
1	14	2.70	18	0.78
2	6.9	1.20	8.2	0.83
3	8.0	1.40	9.6	0.83
4	5.0	1.00	6.9	0.72
5	8.0	1.60	11	0.72
6	4.9	0.80	5.5	0.90
7	4.7	0.75	5.2	0.91
8	5.9	1.10	7.6	0.78
9	5.2	0.90	6.2	0.85
10	5.4	1.95	6.5	0.83
11	5.3	0.85	5.9	0.90
mean ± sd				0.82 ± 0.06

Calculation of wax volume (cm^3) :

$v = \pi r^2 h$

v = volume (cm^3)
r = radius of capillary tube (cm)
h = height of wax 'slug' (cm)

Example : $v = \pi \left[\frac{0.094}{2}\right]^2 \text{x} 2.65 = 0.018\text{cm}^3$

Calculation of wax density (g/cm^3) :

$\rho = \frac{m}{v}$

ρ = density (g/cm^3)

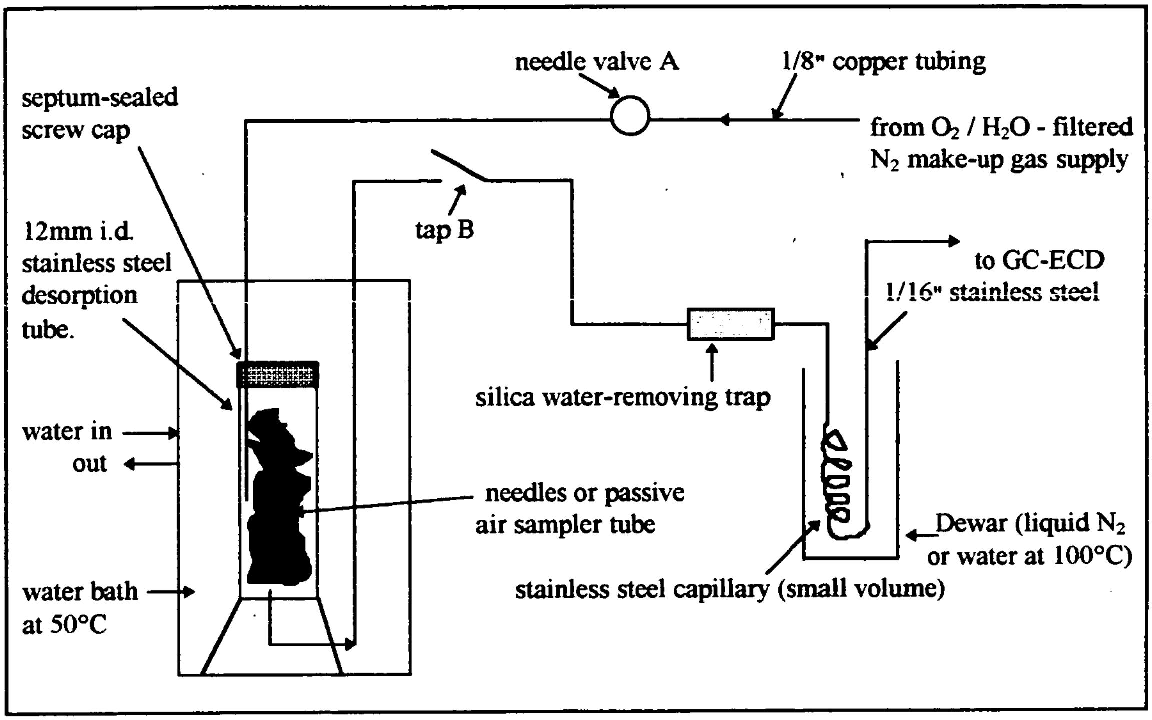
m = mass (g)
v = volume (cm^3)

Example : $\rho = \frac{0.014}{0.018} = 0.78 \text{ g/cm}^3$

∴ Wax density, expressed as mean ± standard deviation (n=11) is 0.82 ± 0.06 g/cm^3

Appendix 3 : Thermal desorption trials

The passive sampler or dried leaves were heated (water bath) to 50°C. The volatiles released were cold trapped (-177°C liquid N_2) and transferred directly to the GC capillary column by rapid heating to 100°C (boiling water) from a stainless steel coil interfaced to the GC injection port via the split vent.



Thermal desorption method

(i) Set up GC/ECD for data collection.
(ii) Blank off injection port with un-pierced septum.
(iii) Set nitrogen flow rate through the thermal desorption system using needle valve (A) and bubble flow-meter. Valve B open.
(iv) Place stainless steel capillary coil in liquid nitrogen Dewar for 10 minutes to equilibrate.
(v) Place sample tube containing wax (air sampler) or foliage sample into the stainless steel desorption vessel and allow to equilibrate outside the water bath (2 minutes) with nitrogen purge to remove any laboratory air in system.
(vi) Place desorption vessel into water bath at 50°C and allow desorption to proceed for 15 minutes. Volatiles will be cold-trapped.
(vii) Turn off nitrogen flow at the needle valve (A) and close tap B (on/off).
(viii) Connect stainless steel capillary column exit to GC split vent (swagelok fittings).
(ix) Replace the liquid nitrogen dewar with one containing boiling water and start GC run (SE-54 column ; oven 30°C for 5 min, 10°C/min to 100°C, 30°C/min to 180°C, 10 min hold time. He @ 6.5 psi. N_2 @ 38.5 ml/min.). The positive pressure generated by the flash vaporisation of the cold-trapped condensed gas sample forces the sample into the injection port and onto the column.
(x) Open valves A and B, purge the system for 10 minutes to flush out remaining volatiles onto the column.

Appendix 4 : The theory of gas chromatography

1. The process

The stationary liquid phase exists as a thin film distributed over an 'inert' granular support (packed column) or supported on the inner column surface of a capillary column. The column is continually swept with the mobile gas phase (carrier gas) at an appropriate temperature. The injected sample components are swept towards the detector whenever they move into the carrier gas stream. Molecules of those components displaying greater affinity for the stationary phase require a greater time to reach the detector than those with less affinity for the stationary phase. Separation of different components is by this differential distribution between the two phases, and the components are distinguished by their different retention times (Willett, 1987). Solute retention time is affected by changing the column temperature or stationary phase, or the diameter/film thickness of the column (Rood, 1991).

2. Mathematical treatment

2.1 During GC, the majority of the transit time of a solute is spent in the mobile gas phase rather than the stationary liquid phase. Effective separation of a mixture depends on the components having significantly different distribution equilibria between these two phases (equation 1).

The **equilibrium distribution** between the two phases is expressed as :

$$K_D = \frac{C_s}{C_m} \qquad (1)$$

where K_D = distribution constant

C_s = concentration per unit volume of stationary (liquid) phase

C_m = concentration per unit volume of mobile (gas) phase

The magnitude of K_D is governed by the solute, temperature and the stationary phase. The gas phase plays a mainly passive role as few molecular interactions can occur. Critical parameters are component vapour pressure (K_D values decrease with increasing temperature and concomitant higher vapour pressure) and sorption to the stationary phase. Different K_D values result in different solute bands on the column. Ideally, a tight, concentrated band upon injection traverses the column and is delivered to the detector as a narrow, sharp peak. In reality, factors such as gaseous dilution resulting from longitudinal diffusion will cause broadening of the solute bands. Normally, with progression down the column, the centres of bands with different K_D values will become increasingly separated; however, band broadening and overlapping of peaks may occur as the tailing edge of the faster component inter-diffuses with the leading edge of the slower analyte.

2.2 Separation efficiency can be expressed in terms of theoretical plates. In static terms, this is the number of 'volatilisation' steps, i.e. the number of times selective desorption and resorption of the solute across the width of the column occurs with progression down the column towards the detector. The process actually happening is dynamic rather than a series of discrete steps. GC column separation efficiency is governed by relative retention and is inversely related to the degree of broadening (function of peak width, *w*) (equation 2).

$$n = 16\frac{(t_R)^2}{w_b} = 5.54\frac{(t_R)^2}{w_{1/2}} = \frac{[(t_R)^2]}{\sigma} \qquad (2)$$

where
$n =$ number of theoretical plates
$t_R =$ time (or distance) from point of injection to peak maximum
$w_b =$ idealised peak width at base line
$w_{1/2}$ ($= w_h$) = width of peak at idealised half height
$\sigma =$ standard deviation of the peak

If a gaussian peak shape is assumed, $w_{1/2} = 2.35\sigma$ and $w_b = 4.00\sigma$.
The values of t_R and w are measured in the same units (seconds or cm), $w_{1/2}$ is measured directly. Non-retained components require a certain volume of carrier gas for complete transit through the column. This is termed the gas-hold up volume, and is proportional to the gas hold-up time (dead time), t_m, i.e. t_m is the length of time (or distance) that every solute spends in the mobile phase irrespective of retention times. The leading edge of a peak produced by an injection of N_2O or another gas with a vanishingly small K_D value at reasonable temperatures can be used to calculate t_m (Figure 2.2.1). As t_m contributes nothing to the separation process, an adjusted retention time, $t_{R'}$ is defined (equation 3).

$$t_{R'} = t_R - t_m \qquad (3)$$

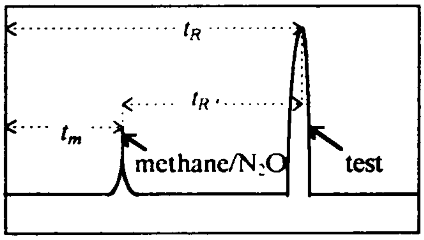


Figure 2.2.1 Experimental determination of t_m

The solute must be in either the gas or liquid phase. The time t_m will be spent in the gas phase irrespective of retention time, therefore by rearrangement of equation (3), $t_{R'}$ is spent in the liquid phase :

$$t_R = t_m + t_{R'} \qquad (4)$$

The number of usable, or **effective, theoretical plates (N)** can now be calculated:

$$N = 4^2 \frac{(t_{R'})^2}{w_b} = 5.54 \frac{(t_{R'})^2}{w_{1/2}} \tag{5}$$

The higher the number of plates, the greater the column potential to separate two peaks. Longer columns - assuming identical efficiencies per unit length - will possess more theoretical plates, although the relationship is only linear at the optimum gas velocity (u_{opt} - see section 2.7) due to pressure drops etc. and the increase in separation (resolution) will only be a function of the square root of the column length. Efficiencies are generally expressed in terms of the length of column occupied by one theoretical plate, ***h*** (mm), the 'shorter' each theoretical plate, the greater the number that can fit into a unit length of column.

$$h = \frac{L}{n} \tag{6}$$

where h = height equivalent to one theoretical plate

L = column length (m)

The smaller the value of ***h***, the more efficient the column and the greater the separating power. When the column is operating at u_{opt}, h_{min} is used to express the value of ***h***. As column temperature, test compound and the nature of the carrier gas affect n (and N), ***h*** is also affected by these parameters. One of the easiest methods to increase column efficiency is by using smaller diameter columns (equation 7); film thickness (d_f) only has a slight effect on efficiency.

$$h_{\min} = r_o \left\{ \frac{11k^2 + 6k + 1}{3(1+k)^2} \right\}^{1/2} \tag{7}$$

where r_o = column radius

k = partition ratio

h_{min} = height equivalent to a theoretical plate at u_{opt}

2.3 The **partition ratio,** ***k***, is an accurate measure of how long a time the molecules of a species spend in the liquid phase relative to their time in the gas phase, and is inversely proportional to column temperature.

$$k = \frac{t_{R'}}{t_m} = \frac{t_R - t_m}{t_m} \tag{8}$$

And:

$$N = n \frac{(k)^2}{k+1} \tag{9}$$

If K_D is very large, and retention times are long, the solute has a very large partition ratio and $k/k + 1$ tends to 1, and N tends to n.

The theoretical relationships that exist between h_{min}, the column radius (r_0) and k are useful (Figures 2.3.(1,2)).

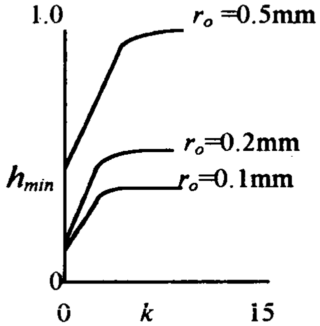


Figure 2.3.1

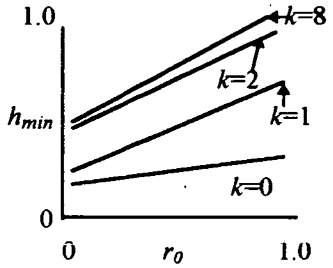


Figure 2.3.2

Figure 2.3.1 shows that the larger the column radius, the higher the h_{min} value, and the less efficient the column. The relationship between r_o (column radius) and h_{min} in Figure 2.3.2 is one of decreasing efficiency with increasing k and r_o.

2.4 The **phase ratio**. The proportion of time that a substance spends in the liquid phase (k) must relate to its distribution coefficient, K_D. This relationship hinges on the relative availability of (volumes occupied by) the gas and liquid phases, i.e. the phase ratio β.

$$\beta = \frac{V_G}{V_L} = \frac{V_m}{V_s} \qquad \text{(dimensionless)} \qquad (10)$$

$V_{G/m}$ = volume occupied by gas / mobile phase
$V_{L/s}$ = volume occupied by liquid / stationary phase

$$\text{As } K_D = \frac{\text{(amount in liquid / volume of liquid)}}{\text{(amount in gas / volume of gas)}} \qquad \text{as in equation (1)}$$

$$= \text{(amount in liquid / amount in gas) x (volume of gas / volume of liquid)}$$

$$= k\beta \qquad (11)$$

β can be considered to be a measure of column 'openness', those of open tubular (or capillary columns) being 50 - 1500, packed columns from 5 - 35. At constant film thickness, V_L is governed by the surface area of capillary columns and varies directly with column diameter. The volume of the column governs V_G and is directly proportional to the square of the inner radius :

$$\beta = \frac{V_G}{V_L} = \frac{V_m}{V_s} = \frac{r_o}{2d_f} \qquad (12)$$

r_o = inner radius : distance from column centre to the liquid phase surface
d_f = column external diameter

The magnitude of the constant K_D (= βk) depends on analyte, column temperature and the liquid phase. The phase ratio, β is solely a function of the diameter and the film thickness of a column, and cannot be altered. For a different column, an increase in β, e.g. increase of r_o or decrease of d_f, will be accompanied by a decrease in k and t_R (equation 8). Faster analysis times result, but n is reduced as less time is spent in the liquid phase. This relationship is independent of other parameters such as carrier gas type, column length and flow rate. Because all solutes are affected proportionally, relative retentions remain the same. Table 2.4.1 summarises the effects of changing column diameter and film thickness on retention.

Table 2.4.1 Effects of changing column diameter and film thickness on retention and efficiency

Column diameter ($2r_o$)	Film thickness (d_f)	Phase ratio (β)	Retention (k)	Efficiency (h, n)
Increased	-	Increases	Decreases	Reduced
Decreased	-	Decreases	Increases	Improved
-	Increased	Decreases	Increases	Reduced
-	Decreased	Increases	Decreases	Improved

Relationships are valid for constant column temperature, stationary phase & solute.
Adapted from Rood, 1991.

Changing β by altering film thickness is the primary means of altering column retention, rather than diameter or length. The main effect of diameter reduction is an improvement in efficiency, increasing the length is of limited use in temperature-programmed runs.

Thicker film columns ($\beta < 100$) are used to retain more volatile compounds allowing an increase in $t_{R'}$, increased interaction with the stationary phase and improved separation at higher temperatures. Disadvantages are higher background bleed and slightly lower efficiency. Thin film columns ($\beta > 400$) are used for high boiling point solutes to minimise analysis time. Standard films (β = 100-400) are satisfactory for most analyses over a reasonable range of volatilities, e.g. 0.25μm for 0.25mm/0.32mm I.D. columns, and 1.0-1.5 μm for 0.53mm I.D. columns.

2.5 Separation factor or selectivity (α) Component separation is achieved as a function of retention time ratios and *n*. The ratio of the adjusted retention times of the two solutes under identical conditions is termed their separation factor,α. This is always equal to or greater than one (equation 13).

$$\alpha_{1,2} = \frac{t'_{R(2)}}{t'_{R(1)}} = \frac{k_{(2)}}{k_{(1)}} = \frac{K_{D(2)}}{K_{D(1)}} \qquad (13)$$

where 1 = earlier eluting peak, and 2 = later eluting peak.

Solute pairs with a large α value can be separated on columns with a small *n*, but as α tends to 1, increasingly larger values of *n* are required. Control of α is by the stationary phase to achieve the greatest relative retention of components.

Resolution is a better measurement of selectivity as it takes into account peak width, i.e. the difference in time between two narrow peaks may be less than for two broad peaks; but superior resolution is observed in the first case.

$$R_s = 2\left[\frac{t_{R2} - t_{R1}}{w_{b2} + w_{b1}}\right] \qquad (14)$$

where t_R = retention time of peaks 1 and 2
w_b = peak width at base of peaks 1 and 2

The greater the R_s value, the greater the separation. A resolution of 1.5 represents 100% resolution (baseline separation). A resolution of 0.75 is reasonable qualitatively but not quantitatively, e.g. severe tailing can occur when minor components are separated from major ones which do not have symmetrical peaks.
If the assumption is made that $w_1 = w_2$ then ;

$$R_s = \frac{n^{1/2}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right) \qquad (15)$$

Thus, R_s is proportional to $n^{1/2}$ (or length of column - a large portion of the column can be removed before a significant reduction in R_s is observed) and also is influenced by *k* and α (selection of stationary phase).
The best method to improve resolution is by manipulation of α for maximum selectivity of the components of interest. Increasing *k* is satisfactory when *k* is small. As *k/k*+1 tends to 1, increases in *k* have little effect on R_s.

2.6 Capacity The capacity of a column is the amount of solute where broadening of $w_{1/2}$ by 10% occurs. Capacity is dependent on column diameter, d_f and the solute - stationary phase polarity match. Increasing the former increases capacity. For a 0.32mm column of 0.25 - 0.50µm film thickness, the capacity is 250 - 600ng per injection (Alltech, 1991; Rood, 1991); that of thicker films is greater.

2.7 Van Deemter equation This equation evaluates the relative importance of a series of parameters on column efficiency. In its abbreviated form :

$$h = A + \frac{B}{u} + Cu \qquad (16)$$

where h = efficiency (height equivalent to one theoretical plate)

u = average linear velocity of the mobile phase (carrier gas) in cm/sec

$$= \frac{L}{t_m} \qquad (17)$$

A = packing/multiflow factor term

B = longitudinal diffusion term

C = resistance to mass transfer

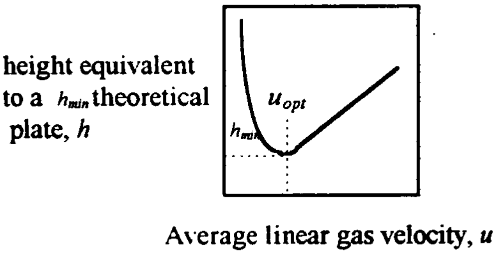
L = column length (cm)

t_m = retention time of unretained solute (sec)

The aim is to reduce the h term (highest n, or N) commensurate with the degree of separation required, the range of k-values of solutes and the analysis time required. In capillary columns, A is eliminated accounting for the increased efficiency observed :

$$h = \frac{B}{u} + Cu \qquad \text{Golay equation} \qquad (18)$$

Capillary columns additionally have much larger β values, and much longer columns can be used before the pressure drop becomes limiting. There is less departure from uniformly thin film and cross-sectional inhomogenity in temperature contributing to band broadening. The optimum gas velocity (u_{opt}) can be identified as that value of u (expressed as an average, as it is slower in the latter part of the column) which results in greatest column efficiency for a given k-value under defined conditions.



The system generates the maximum number of n per unit time at the optimum practical gas velocity (OPGV). Using a flow that is too high or too low will result in substantial loss of efficiency even when all other parameters have been suitably chosen [L, r_o, d_f, mobile phase, temperature, stationary phase (through effects on α and k)]. The increase in carrier gas viscosity, with consequent decrease in flow rate, observed with increase in temperature during a temperature-programmed run necessitates use of the OPGV (1.25-2 x u_{opt}). If , at a constant inlet pressure, the flow rate is set at the exact minimum in the Van Deemter curve, a concomitant decrease in efficiency will be observed.

The three parameters to be evaluated when using Van Deemter curves are h_{min}, u_{opt} (OPGV and analysis time vary directly with u_{opt}) and the 'flatness' of the curve. Van

Deemter plots are *k*-specific, and the range of *k*-values over which one gas velocity can reasonably be 'optimal' varies with flatness of curve (Figure 2.7.1).

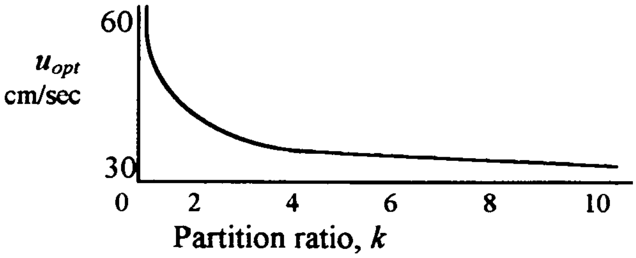


Figure 2.7.1 The optimum average linear gas velocities (μ_{opt}) as a function of k on a 30m x 0.25mm column (d_f = 0.25μm, He carrier).

The effects of increasing film thickness, r_o and L on Van Deemter curves for a given solute at known k using helium as a carrier gas are as Figure 2.7.2, the change in u_{opt} is less marked :

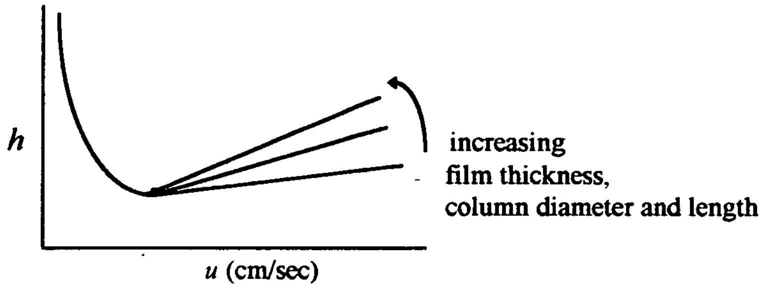


Figure 2.7.2 In general, as column diameter, length and film thickness increase, the steepness of the corresponding Van Deemter curve increases (Rood, 1991).

The choice of carrier gas is important. For He and H_2, the minimum in the efficiency curves occurs over a much broader range and at higher linear velocities than nitrogen (Figure 2.7.3; Rood, 1991). Using N_2 will result in the greatest efficiency, but increasing u results in a sharp drop in h, h_{min} occurring over a very narrow range at low linear velocities. Substantial analysis speed must be sacrificed for optimal R_s.

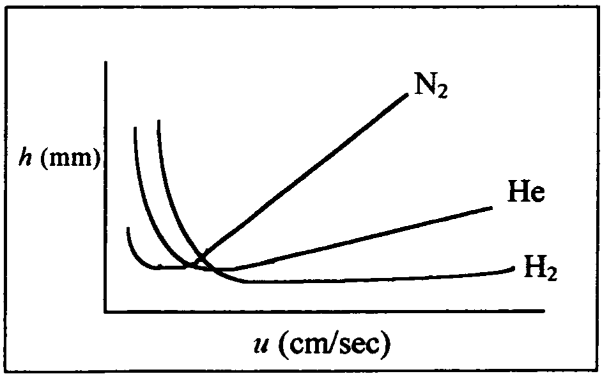


Figure 2.7.3 Van Deemter curves: 30m x 0.25mm ID, 0.25μm film capillary column (adapted from Rood, 1991).

Using He, and particularly H_2, allows for high flow rates with fast analysis times without sacrificing a large amount of separation efficiency, even over a wide range of solute volatilities. Large changes in oven temperature will not cause gross fluctuations in column efficiency. The u_{opt}, OPGV values and flow rates for He and H_2 are given in Table 2.7.1. An increase in k (longer $t_{R'}$) results in higher h_{min} values and an increase in curve steepness. With extremely thick films, high k-solutes finally exhibit lower values for h_{min} than do low-k solutes. The various cross-over points are a function of d_f and not β. **Volumetric flow rate** is calculated as equation 19;

$$F = \frac{\pi r_o^2 L}{t_m} \tag{19}$$

where F = volumetric flow rate (ml/min)

Table 2.7.1 Approximate μ_{opt}, OPGV and flow rates ($k = 7$) on a 30m column.

Diameter (mm)	Linear velocity (cm/sec)		OPGV (cm/sec)		Flow rate (ml/min)	
	He	H_2	He	H_2	He	H_2
0.25	31	54	45	78	0.9-1.3	1.3-1.8
0.32	33	55	48	84	1.4-2.2	1.3-1.8
0.53	31	42	61	101	4.0-6.0	6.0-7.9

From Rood, 1991 and J & W Scientific press

The **velocity window concept** allows the analyst to decide the acceptable maximum increase in h to be tolerated at the two limits of interest in the chromatogram - the lowest and highest k-values - between which a high degree of separation is required. The window is defined by u corresponding to the upper k (low u_{opt}) and lower k (high u_{opt}) values. Figure 2.7.4 shows a permitted 10% increase in h_{min}.

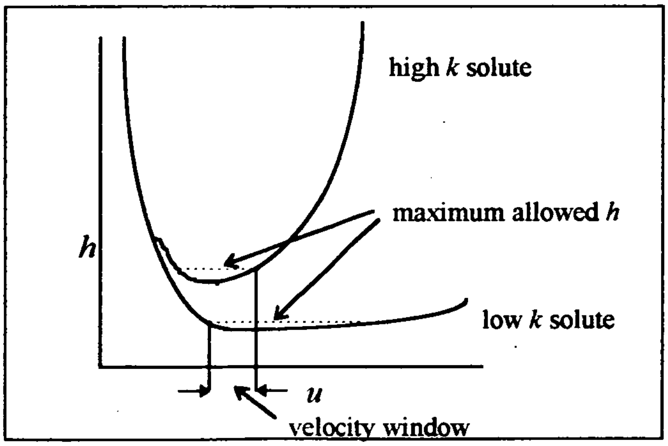


Figure 2.7.4 The velocity window concept

The dramatic effects of using different carrier gases on the possible velocity windows are as Figure 2.7.5. N_2 is obviously of least use, as the Van Deemter curve is steeper, the window is very narrow at low velocities. If no such window exists for the conditions specified, the analysis must be limited to a lower range of solutes or a greater increase in *h* permitted.

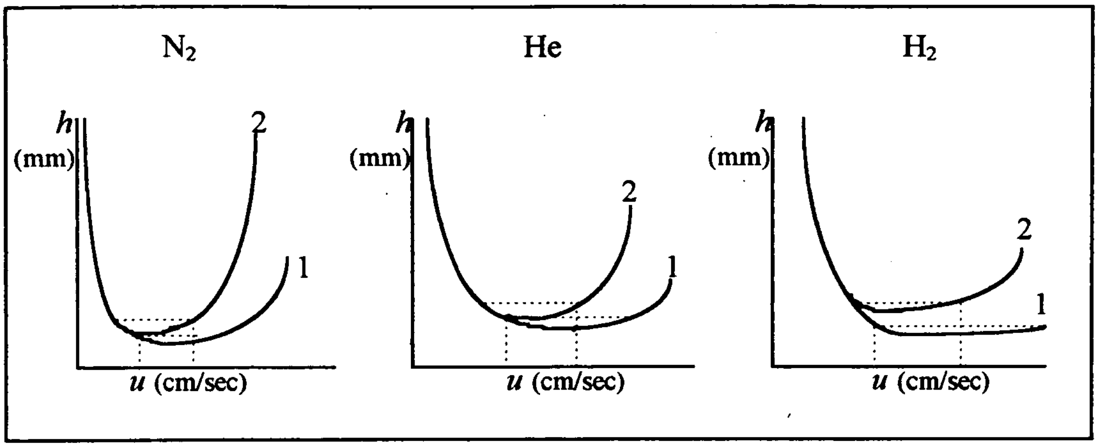


Figure 2.7.5 Van Deemter curves for different carrier gases. The difference between N_2 and H_2 is marked. Curve 1 ($k = 0.5$) and curve 2 ($k = 10$) demonstrate the *k*-specific nature of Van Deemter plots.

The optimum conditions can be predicted for a given separation following choice of carrier gas and column characteristics. The t_m and the *k*-values are determined and the velocity window and OPGV for maximum efficiency and resolution adopted.

3 Terminology

It is useful to define a number of terms relating to quantitation :

3.1 Sensitivity (response)

For a GC detector, the sensitivity is the product of the peak area and flow rate divided by the sample weight (g, moles). The halogen response order of an ECD is I>Br>Cl>F. An increase in response is also seen for multiple halogen substitution, especially on the same C atom. This suggests that $CH_3I>CCl_4>C_2Cl_4>CHCl_3>C_2H_3Cl_3>C_2HCl_3$, an order confirmed in practice (Table 3.1.1). For multiple halogen substituted compounds, amounts of less than 1 pg can be detected (Rood, 1991).

Table 3.1.1 Experimental ECD sensitivity to halogen-containing molecules based on mean of all 1µl injections of standard over all time at a flow rate of 1.03ml/min.

Elution order	Peak area of 180pg/µl standard (µV.s)	Sensitivity (area counts, mV/(µg/m^3))	Relative sensitivity (to C_2HCl_3)
CH_3I	36.8×10^6	3.51	28.5
$CHCl_3$	4.03×10^6	0.38	3.1
$C_2H_3Cl_3$	2.61×10^6	0.25	2.0
CCl_4	12.0×10^6	1.14	9.3
C_2HCl_3	1.29×10^6	0.12	1.0
C_2Cl_4	4.60×10^6	0.44	3.6

3.2 Linear range

For a ^{63}Ni ECD detector, the change in concentration of a compound in an injected sample results in a proportional change in the peak size only over the linear range of the detector. If the concentration of the compound is outside this range, the detector response is not proportional to the amount of compound passing through the detector. The linearity of the detector is determined by plotting the response peak size Vs. compound concentration for a series of standard mixes. The minimum concentration used to generate the linear range plot should be at the detection limit. The literature upper component concentration for the linear range is 0.5-1ng per injection depending on geometry of the detector and column flow rate (Rood, 1991).

3.3 Total uncertainty

The total uncertainty is a combination of the bias and precision (systematic and random errors) (Black, 1991) :

$$U_T = \left(S_T^2 + \tfrac{1}{3}B^2\right)^{1/2}$$

where U_T = total uncertainty
S_T = total random error (precision)
B = systematic error (bias)

The 95% level is used , as the bias is known with high confidence (Table 3.3.1) being obtained from spiking experiments to determine % recovery. The total uncertainty value sets the interval in which there is a 95% probability that the true value lies. The total uncertainty is calculated here to estimate uncertainties in experimental data.

Accuracy is a combination of precision and bias where precision is high and bias low. Any other combination is inaccurate.

Table 3 3.1 Uncertainty calculation data based on 3 injections of standards containing 180pg/µl into foliage.

Chlorinated solvent	Spiking recovery (x) (%)	Precision at 95% level = 2 x sd% of recovery	Bias x-100%	U_T
$CHCl_3$	128	±20%	28%	26%
$C_2H_3Cl_3$	132	±18%	32%	26%
CCl_4	115	±0.2%	15%	7%
C_2HCl_3	110	±10%	10%	12%
C_2Cl_4	108	±12%	8%	13%

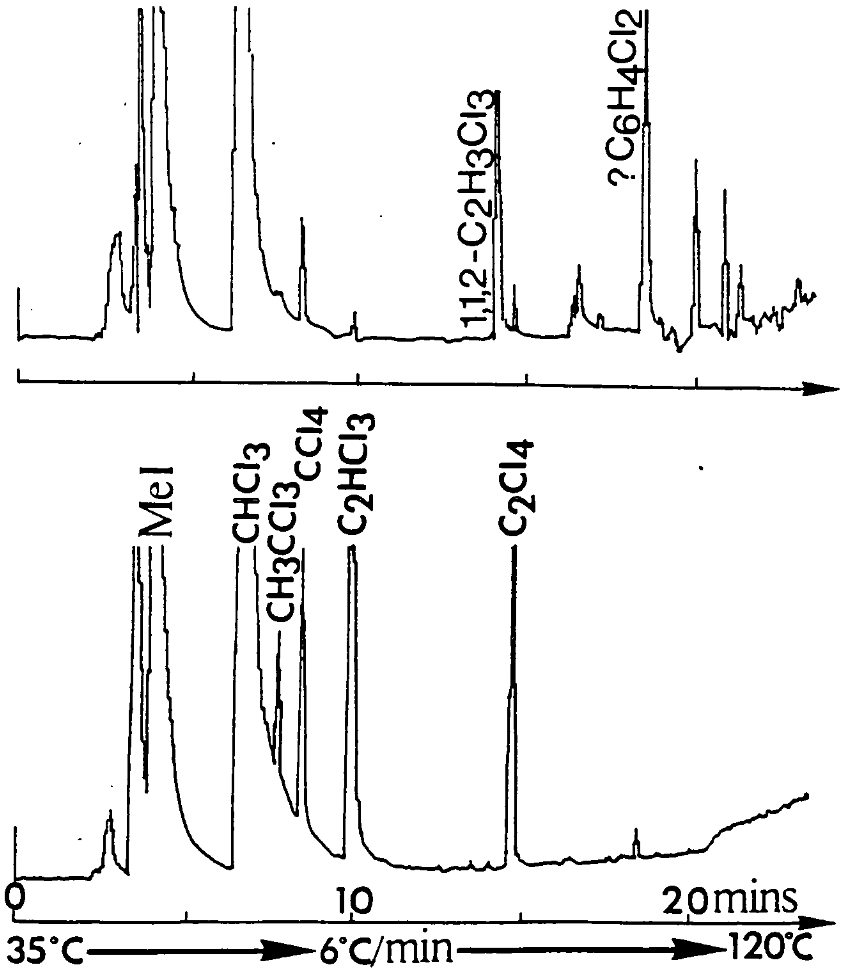
Appendix 5 : Example gas chromatographic trace

An example GC/ECD run of a calibration standard is shown (180pg/injection), and also the chromatogram from a sample run (Banchory forest, near Crathes).
The optimised GC/ECD conditions used for all calibration and sample analyses were :

Column :	SE-54 (30m x 0.32mm ID, 1µm film) + retention gap (1m x 0.25mm ID, deactivated silica). Temperature ramp : 35°C 8 min hold, 6°C/min to 120°C, 30°C/min to 290°C, 10 min hold
Carrier :	He @ 6.4 psi
Injector :	Splitless, 150°C; 1µl injection volume
Detector :	ECD @ 375°C; 45ml/min N_2 makeup gas

Example gas chromatogram

Sample : Crathes 1992 year class needles, 10m height, collected November 1993



standard run for retention time comparison

Appendix 6 : Determination of the conversion ratio from needle dry weight to surface area for a typical batch of needles

All the results in Chapters 6 and 7 are expressed as ng/g dry weight. An indicative measurement of the relationship between leaf surface area and dry weight was made for the needles used in this study so that an approximate conversion factor could be obtained and data may be compared with other results expressed in terms of leaf area. Such a conversion factor is also important because needle size differed at different sites and it may be more appropriate to compare on an area basis rather than dry weight, i.e. if most of the chlorinated solvent is held in the outer wax layer, it may be more appropriate to express loading in terms of surface area rather than dry weight of needles.

Method

A sub-sample of 15 individual fresh needles from each forest were measured (digital micrometer) and weighed. The needles were oven dried for 72 hours at 80°C and then individually re-weighed. The needle dry weight and size measurements made are given in Table 1-4.

The surface area and volume for each individual needle (*a*) was calculated from the measurements of needle length, breadth and thickness (Cape, 1986).

The density, ρ, (g/cm^3) was calculated on both a dry weight and fresh weight basis for each individual needle. The mean results averaged over all 60 needles are below :

mean ρ (g fresh weight/cm^3) = 0.889 ± 0.147
mean ρ (g dry weight/cm^3) = 0.373 ± 0.068

Table 1 Dry weight (*w*) and size measurements for a sub-sample of needles collected from Banchory forest at 10m on 25/03/93. Surface area, volume and density calculations for each individual needle are given.

Needle	Fresh weight (g)	Dry weight (g)	Length (mm)	Breadth (mm)	Thickness (mm)	Surface area based on cylindrical leaf shape (cm^2)	Volume based on cylindrical leaf shape (cm^3)	Density (g fresh weight/cm^3)	Density (g dry weight/cm^3)
1	0.0323	0.014	37.11	1.36	0.64	1.50	0.0323	1.000	0.433
2	0.0512	0.022	41.90	1.67	0.80	2.10	0.0560	0.914	0.393
3	0.0416	0.018	36.56	1.73	0.87	1.93	0.0550	0.756	0.327
4	0.0416	0.018	34.08	1.79	0.80	1.79	0.0488	0.852	0.369
5	0.0442	0.019	37.30	1.64	0.74	1.80	0.0453	0.976	0.419
6	0.0392	0.016	32.79	1.78	0.82	1.74	0.0479	0.818	0.334
7	0.0436	0.017	37.78	1.87	0.68	1.95	0.0480	0.908	0.354
8	0.0345	0.017	40.08	1.50	0.70	1.78	0.0421	0.819	0.404
9	0.0289	0.014	27.51	1.76	0.79	1.43	0.0382	0.757	0.367
10	0.0422	0.019	36.39	1.77	0.75	1.86	0.0483	0.874	0.393
11	0.0416	0.018	39.19	1.73	0.66	1.90	0.0447	0.931	0.403
12	0.0518	0.021	39.60	1.85	0.81	2.14	0.0593	0.874	0.354
13	0.0270	0.013	31.52	1.48	0.62	1.34	0.0289	0.934	0.450
14	0.0385	0.016	40.07	1.41	0.61	1.64	0.0345	1.120	0.464
15	0.0464	0.019	39.32	1.58	0.75	1.86	0.0345	1.345	0.551

Table 2 Dry weight (*w*) and size measurements for a sub-sample of needles collected from Ponsonby Tarn forest at 10m on 01/04/93. Surface area, volume and density calculations for each individual needle are given.

Needle	Fresh weight (g)	Dry weight (g)	Length (mm)	Breadth (mm)	Thickness (mm)	Surface area based on cylindrical leaf shape (cm^2)	Volume based on cylindrical leaf shape (cm^3)	Density (g fresh weight/cm^3)	Density (g dry weight/cm^3)
1	0.0191	0.007	38.98	1.07	0.59	1.31	0.0246	0.776	0.285
2	0.0115	0.005	23.17	1.18	0.53	0.81	0.0145	0.793	0.345
3	0.0179	0.006	34.86	1.23	0.53	1.24	0.0227	0.789	0.264
4	0.0119	0.005	23.82	1.09	0.56	0.80	0.0145	0.821	0.345
5	0.0226	0.008	35.84	1.29	0.58	1.36	0.0268	0.843	0.299
6	0.0133	0.005	31.45	1.03	0.50	0.97	0.0162	0.821	0.309
7	0.0192	0.007	34.71	1.41	0.55	1.38	0.0269	0.714	0.260
8	0.0189	0.007	31.40	1.25	0.57	1.23	0.0224	0.844	0.313
9	0.0204	0.007	39.81	1.41	0.53	1.56	0.0298	0.685	0.235
10	0.0160	0.007	42.04	1.19	0.28	1.24	0.0140	1.143	0.500
11	0.0192	0.007	37.51	1.15	0.53	1.27	0.0229	0.838	0.306
12	0.0166	0.006	34.16	1.09	0.46	1.07	0.0171	0.971	0.351
13	0.0135	0.005	31.28	1.01	0.53	0.98	0.0167	0.808	0.299
14	0.0206	0.008	39.73	1.36	0.55	1.53	0.0297	0.694	0.269
15	0.0169	0.008	28.84	1.27	0.57	1.08	0.0209	0.809	0.383

Table 3 Dry weight (*w*) and size measurements for a sub-sample of needles collected from Devilla forest at 10m on 15/03/93. Surface area, volume and density calculations for each individual needle are given.

Needle	Fresh weight (g)	Dry weight (g)	Length (mm)	Breadth (mm)	Thickness (mm)	Surface area based on cylindrical leaf shape (cm^2)	Volume based on cylindrical leaf shape (cm^3)	Density (g fresh weight/cm^3)	Density (g dry weight/cm^3)
1	0.0148	0.006	30.43	1.09	0.44	0.94	0.0146	1.014	0.411
2	0.0170	0.008	30.29	1.35	0.47	1.12	0.0192	0.885	0.417
3	0.0151	0.007	36.72	1.00	0.44	1.07	0.0162	0.932	0.432
4	0.0183	0.009	32.75	1.34	0.51	1.23	0.0224	0.817	0.402
5	0.0285	0.012	37.70	1.39	0.56	1.49	0.0293	0.973	0.410
6	0.0228	0.011	35.87	1.41	0.58	1.45	0.0293	0.778	0.375
7	0.0153	0.007	30.41	1.55	0.43	1.22	0.0203	0.754	0.345
8	0.0197	0.009	34.55	1.38	0.49	1.31	0.0234	0.842	0.385
9	0.0150	0.006	28.12	1.27	0.52	1.02	0.0186	0.807	0.323
10	0.0175	0.008	36.61	1.30	0.52	1.35	0.0247	0.709	0.324
11	0.0248	0.012	36.03	1.42	0.54	1.43	0.0276	0.899	0.435
12	0.0124	0.006	26.66	1.11	0.43	0.83	0.0127	0.976	0.472
13	0.0206	0.009	36.85	1.27	0.42	1.26	0.0197	1.046	0.457
14	0.0169	0.008	33.26	1.23	0.60	1.23	0.0245	0.690	0.327
15	0.0146	0.007	24.70	1.12	0.47	0.80	0.0130	1.123	0.538

Table 4 Dry weight (*w*) and size measurements for a sub-sample of needles collected from Devilla forest at 13m on 15/03/93. Surface area, volume and density calculations for each individual needle are given.

Needle	Fresh weight (g)	Dry weight (g)	Length (mm)	Breadth (mm)	Thickness (mm)	Surface area based on cylindrical leaf shape (cm^2)	Volume based on cylindrical leaf shape (cm^3)	Density (g fresh weight/cm^3)	Density (g dry weight/cm^3)
1	0.031	0.013	37.95	1.64	0.62	1.74	0.0386	0.803	0.337
2	0.0213	0.008	32.21	1.40	0.50	1.24	0.0225	0.947	0.356
3	0.0358	0.015	40.20	1.57	0.58	1.75	0.0366	0.978	0.410
4	0.0370	0.016	39.37	1.60	0.65	1.79	0.0409	0.905	0.391
5	0.0220	0.009	32.49	1.50	0.52	1.33	0.0253	0.870	0.356
6	0.0317	0.013	43.13	1.60	0.55	1.87	0.0380	0.834	0.342
7	0.0171	0.006	32.67	1.15	0.60	1.16	0.0225	0.671	0.267
8	0.0295	0.012	32.82	1.46	0.65	1.40	0.0310	0.952	0.387
9	0.0291	0.013	29.44	1.47	0.64	1.26	0.0277	1.054	0.469
10	0.0286	0.014	36.40	1.88	0.58	1.81	0.0397	0.720	0.353
11	0.0320	0.013	32.60	1.79	0.66	1.62	0.0385	0.831	0.338
12	0.0326	0.012	44.23	1.51	0.58	1.87	0.0387	0.842	0.310
13	0.0116	0.005	25.60	1.02	0.50	0.79	0.0131	0.885	0.382
14	0.0302	0.010	30.11	1.48	0.56	1.25	0.0250	1.208	0.400
15	0.0205	0.007	27.47	1.26	0.43	0.94	0.0149	1.376	0.470

A good linear relationship was found at each site between individual needle dry weight (*w*) and surface area (*a*) for the sub-sample taken. The regression data is given in Table 5, needle dry weight being the (independent) *x* variable. As expected, a linear relationship ($r^2 = 0.81$) was also seen when the sub-sampled data was combined over all the sites (Figure 1).

Table 5 Regression statistics for each site, and the conversion ratio between results expressed as ng/ g dry weight (dwt) and ng/ cm^2 for a typical batch of 50 needles (see example of conversion factor calculation below).

Forest	**Sample date**	**Slope (*m*) (cm^2/g.dwt)**	**Intercept (*c*) (cm^2)**	**Typical ratio for batch of 50 needles (cm^2/g.dwt)**	**Needle dry weight per vial (g) (*W*)**	**Needle area/vial (dry wt x ratio, cm^2)**
Banchory	25/03/93	83±8	0.3±0.1	109	0.572 ±0.022	63
Ponsonby	01/04/93	114±20	0.4±0.2	170	0.360 ±0.018	61
Devilla 10m	15/03/93	95±16	0.4±0.1	126	0.640± 0.039	81
13m	15/03/93	99±13	0.4±0.2	140	0.491± 0.037	69

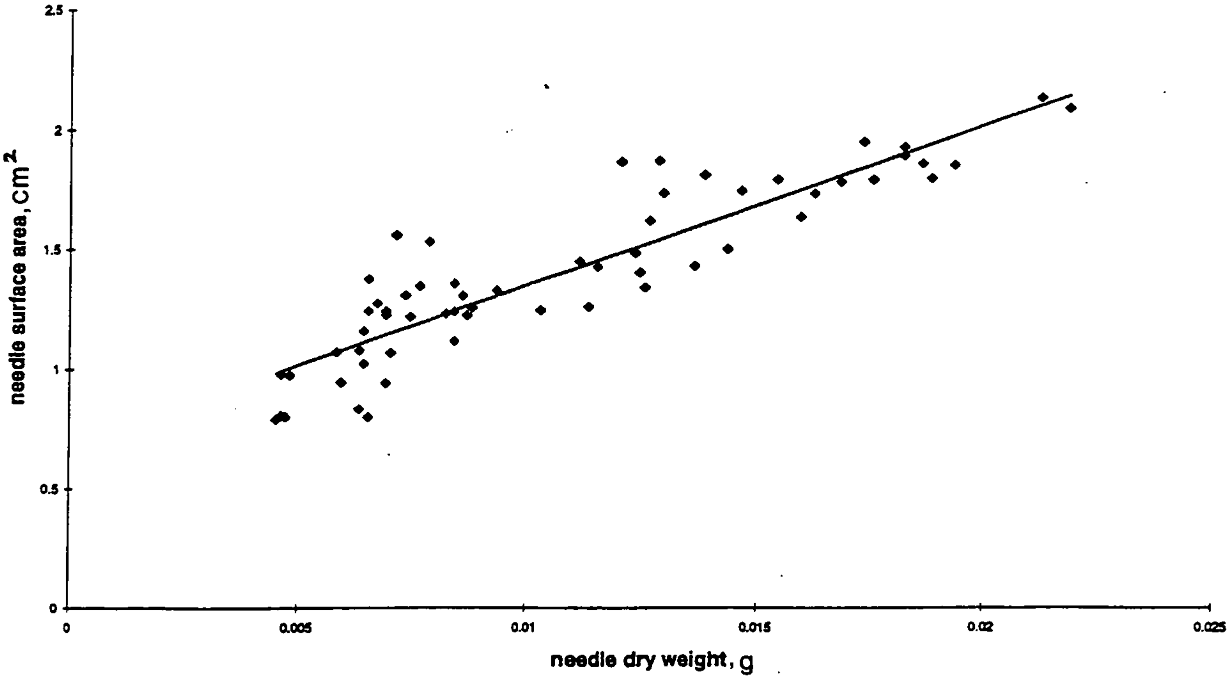


Figure 1 Individual needle surface area plotted Vs dry weight over all three forests

The regression slope and intercept from each site (Table5) were then used to estimate the total surface area (A) of the 50 needles collected in a sample vial :

$$
\begin{aligned}
a &= mw + c \\
A &= 50a = 50(mw + c) \\
&= m\,(50w) + 50c \\
&= mW + 50c \\
&= m'W
\end{aligned}
$$

Where
a = surface area of individual needle (cm^2)
m = slope ($cm^2/g_{dry\ wt}$)
(ratio of dry weight to area for individual needle)
w = weight of individual needle (g)
c = intercept (cm^2)
W = dry weight of vial batch of needles (g) (Table 5)
m' = effective ratio of surface area to dry weight for a sample of 50 needles

Example calculation for Banchory forest

$A = (83 \times 0.572) + (50 \times 0.3) = 62.5\ cm^2$
Typical conversion ratio for a batch of needles = $62.5/0.572 = 109\ cm^2/g_{dry\ wt}$

If the concentration of a solvent is, say, $10ng(g_{dry\ weight})^{-1}$, this is equivalent to $10ng/109cm^2 = 92\ pg/cm^2$

Appendix 7 : Gas chromatography/mass spectroscopy data

The final GC/MSMS method was :

GC
Column SE54 (30m x 0.32mm ID x 1µm film)
Carrier helium @ 7.0 psi
Oven 38°C for 12 mins to 250°C @ 25°C/min for 5 mins.
Injector 38°C for 0.1 min to 250°C @ 140°C/min for 23 mins.
Transfer line @ 250°C
SPI on-column injection of 1µl

MS
Argon = 1-2 mtorr (varied over run)
(1 x 10^{-5} torr manifold pressure)
m/z 61 $C_2H_3Cl_3$
m/z 82 CCl_4
m/z 83 artefact
m/z 95 C_2HCl_3
m/z 129 C_2Cl_4

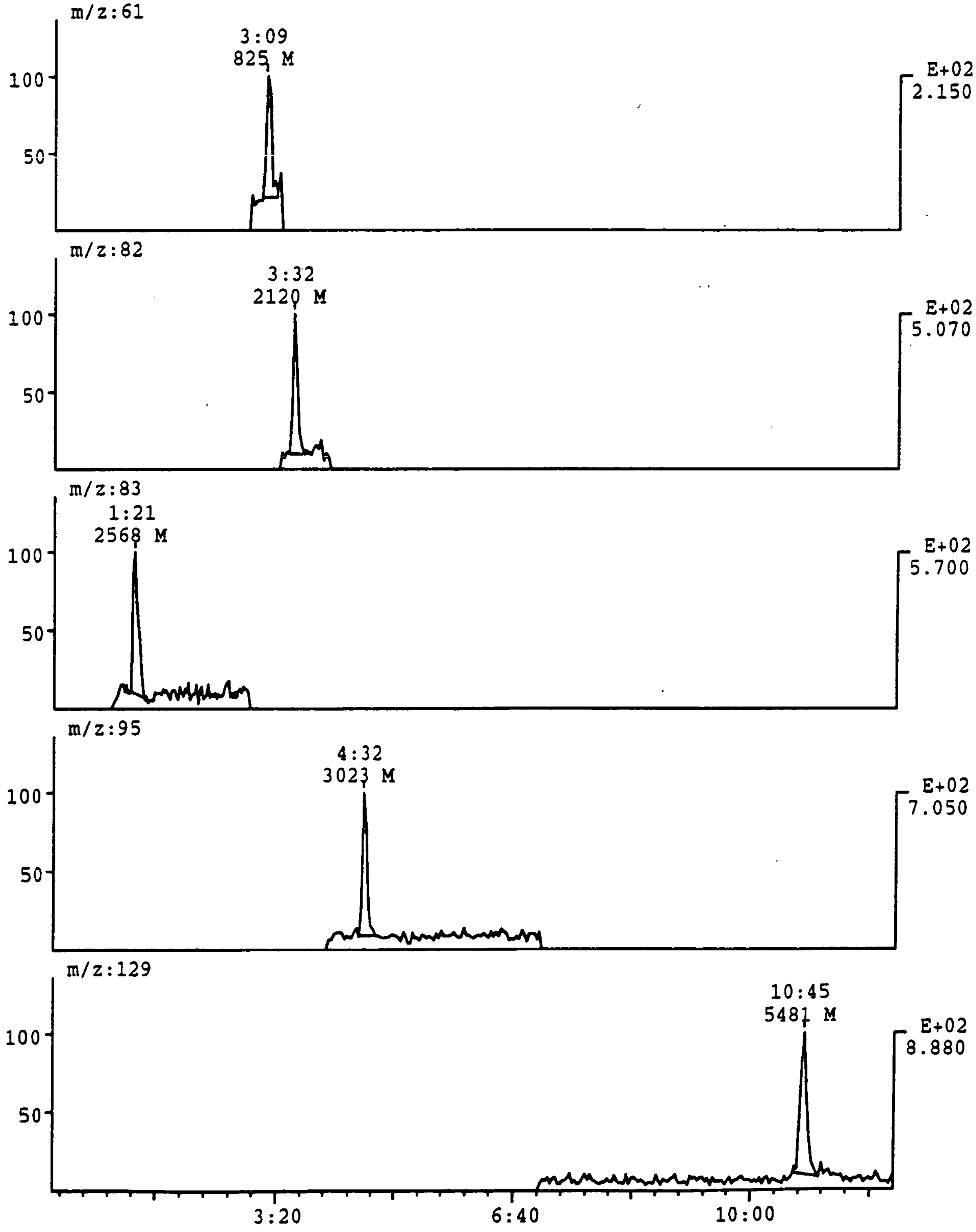
Run procedure for each sampling date
Calibration standards (0.025/0.05/0.5 µg/ml)
Pentane x1
Field blank x1 (refer to Chapter 5)
Samples x1 each

The data in pages rba02-rba10 show a complete run procedure for 1992 and 1993 needles sampled from Devilla forest at 13m and 10m on 21/04/94, comprising :

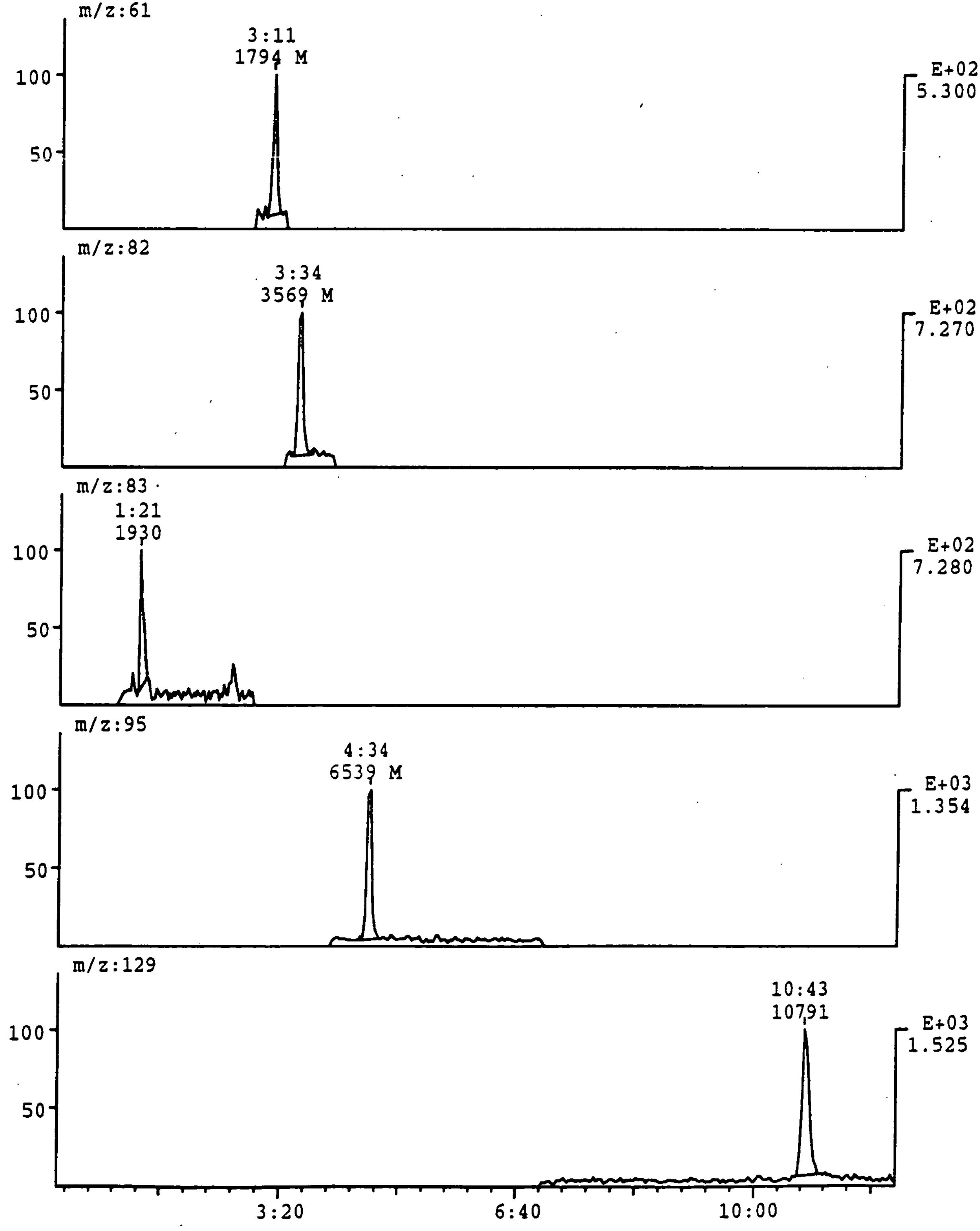
Run number	Sample
rba02	0.025 µg/ml standard
rba03	0.05 µg/ml standard
rba04	0.5 µg/ml standard
rba05	pentane (extraction solvent)
rba06	field blank (Devilla 21/04/94)
rba07	1992 needle extract (13m)
rba08	1993 needle extract (13m)
rba09	1992 needle extract (10m)
rba10	1993 needle extract (10m)

All the calibration data collected using this method is summarised in the table following the GC/MSMS traces. This table is followed by the calculations of C_2HCl_3 (m/z = 95) concentrations present in the Devilla needles sampled on 21/04/94.

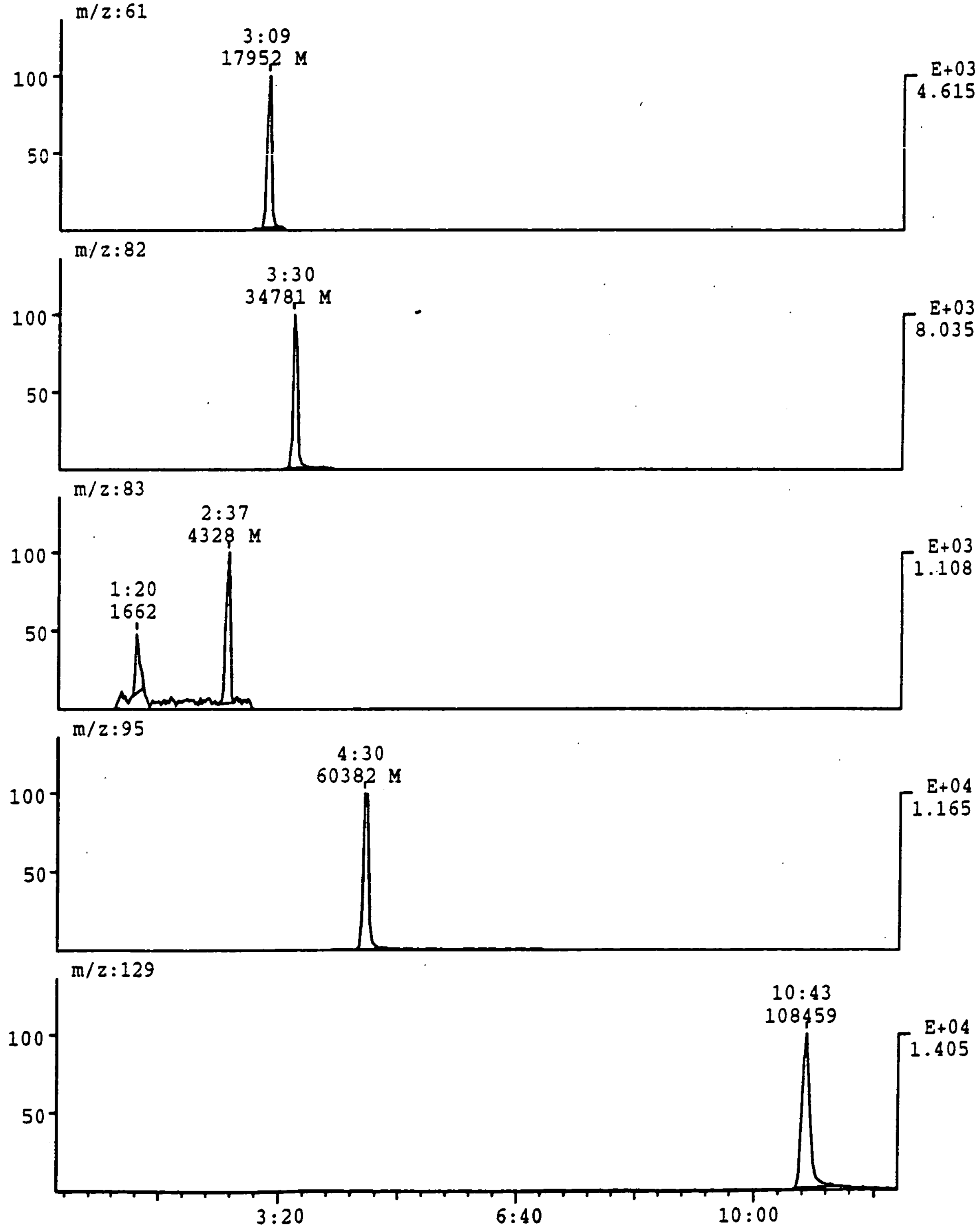
```
CHRO:  rba02                                 04-MAY-94  Elapse:  00:01:37.3    58
Samp:  0.025ug/ml std                                   Start :  13:05:13     377
Comm:  SE- 54 30m 0.32mmid 1um film he 7psi  seq no 2 022/004
Mode:  EI +DAU LMR GAS UP LR                            Study :  Halocarbons
Oper:  A.Newton      Client:  Ruth Brown                Inlet :  GC      Vial   2
Peak:  1000.00  mmu    Label wndw:   1 > 377            Masses:  61 > 129
Area:  2, 4.00         Baseline  :   0, 3               Label :  2, 10.00
Disp:  Area
```



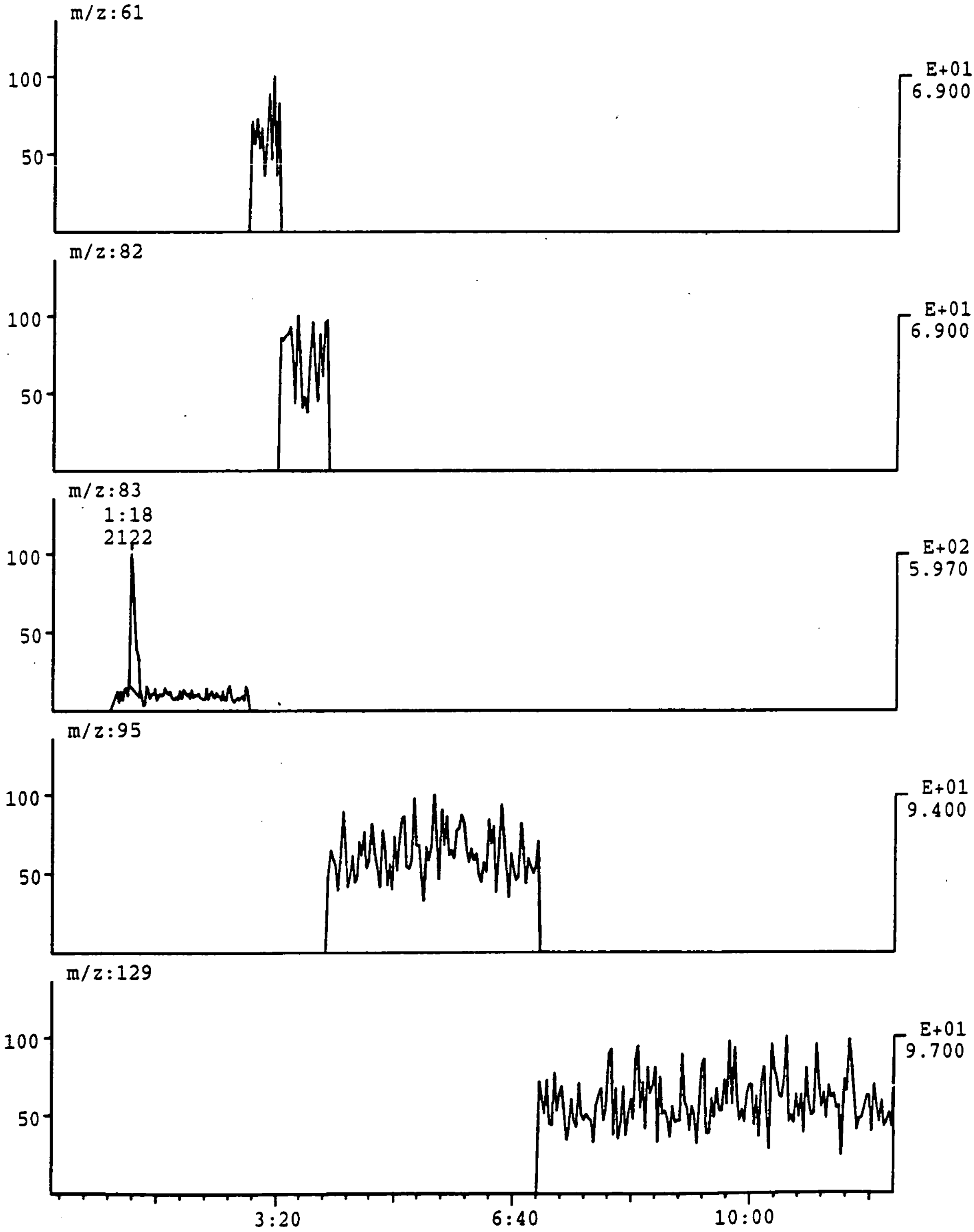
```
CHRO:  rba03                              04-MAY-94  Elapse:  00:11:59.8   377
Samp:  0.05ug/ml std                                 Start :  13:29:02     377
Comm:  SE- 54 30m 0.32mmid 1um film he 7psi  seq no 2 022/004
Mode:  EI +DAU LMR GAS UP LR                         Study :  Halocarbons
Oper:  A.Newton      Client:  Ruth Brown             Inlet :  GC     Vial   3
Peak:  1000.00  mmu    Label wndw:   1 > 377         Masses:  61 > 129
Area:  2, 4.00         Baseline  :   0, 3            Label :  2, 10.00
Disp:  Area
```



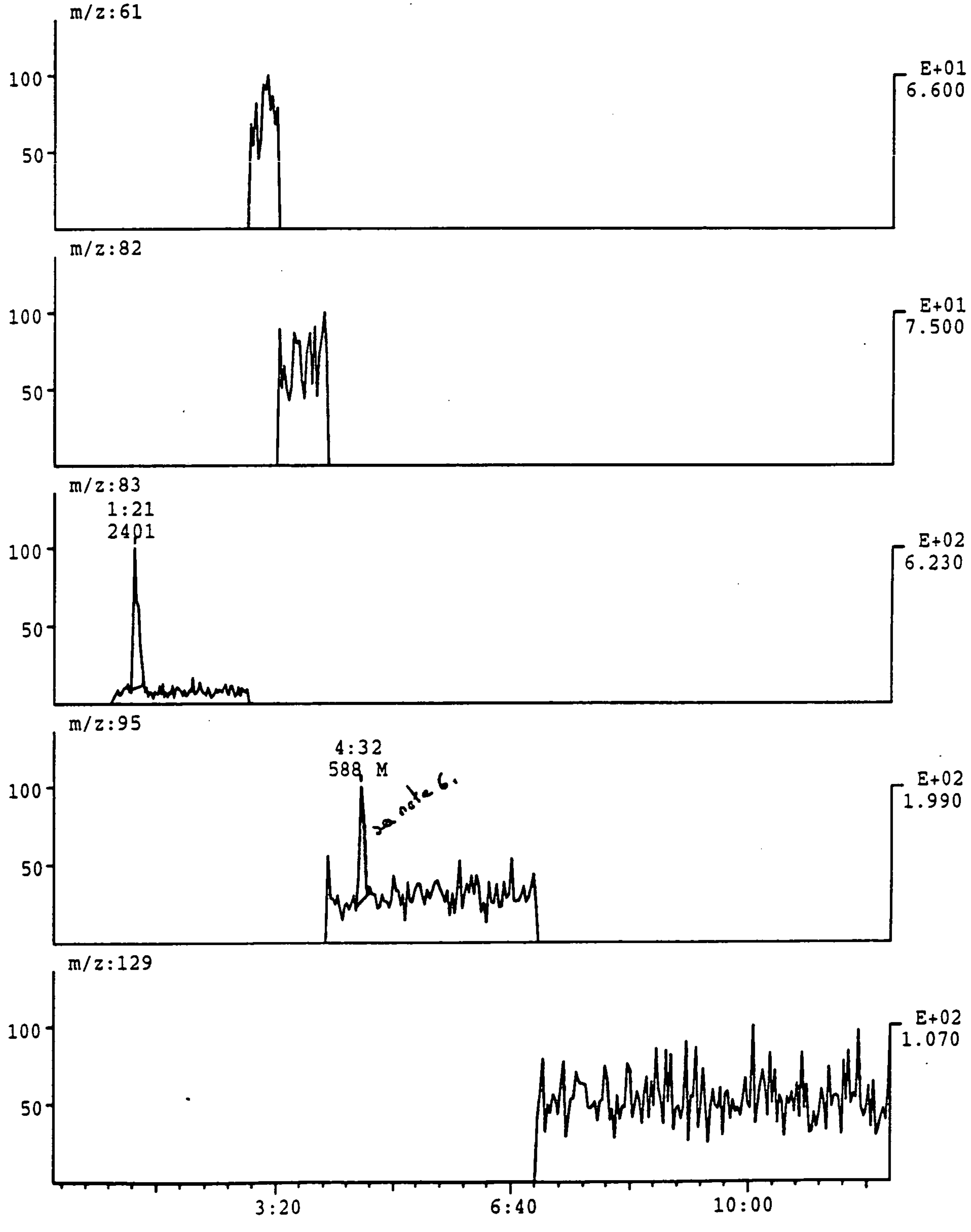
```
CHRO:  rba04                           04-MAY-94  Elapse:  00:02:13.2   84
Samp:  0.5ug/ml std                               Start :  13:52:23    377
Comm:  SE- 54 30m 0.32mmid 1um film he 7psi  seq no 2 022/004
Mode:  EI +DAU LMR GAS UP LR                      Study :  Halocarbons
Oper:  A.Newton      Client:  Ruth Brown          Inlet :  GC     Vial   4
Peak:  1000.00  mmu    Label wndw:   1 > 377      Masses:  61 > 129
Area:  2, 4.00         Baseline  :   0, 3         Label :  2, 10.00
Disp:  Area
```



```
CHRO:  rba05                             04-MAY-94  Elapse:  00:06:37.1   221
Samp:  pentane                                      Start :  14:16:42     377
Comm:  SE- 54 30m 0.32mmid 1um film he 7psi  seq no 2 022/004
Mode:  EI +DAU LMR GAS UP LR                        Study :  Halocarbons
Oper:  A.Newton      Client:  Ruth Brown            Inlet :  GC      Vial   5
Peak:  1000.00  mmu    Label wndw:   1 > 377        Masses:  61 > 129
Area:  3, 3.00         Baseline  :   0, 3           Label :  2, 5.00
Disp:  Area
```

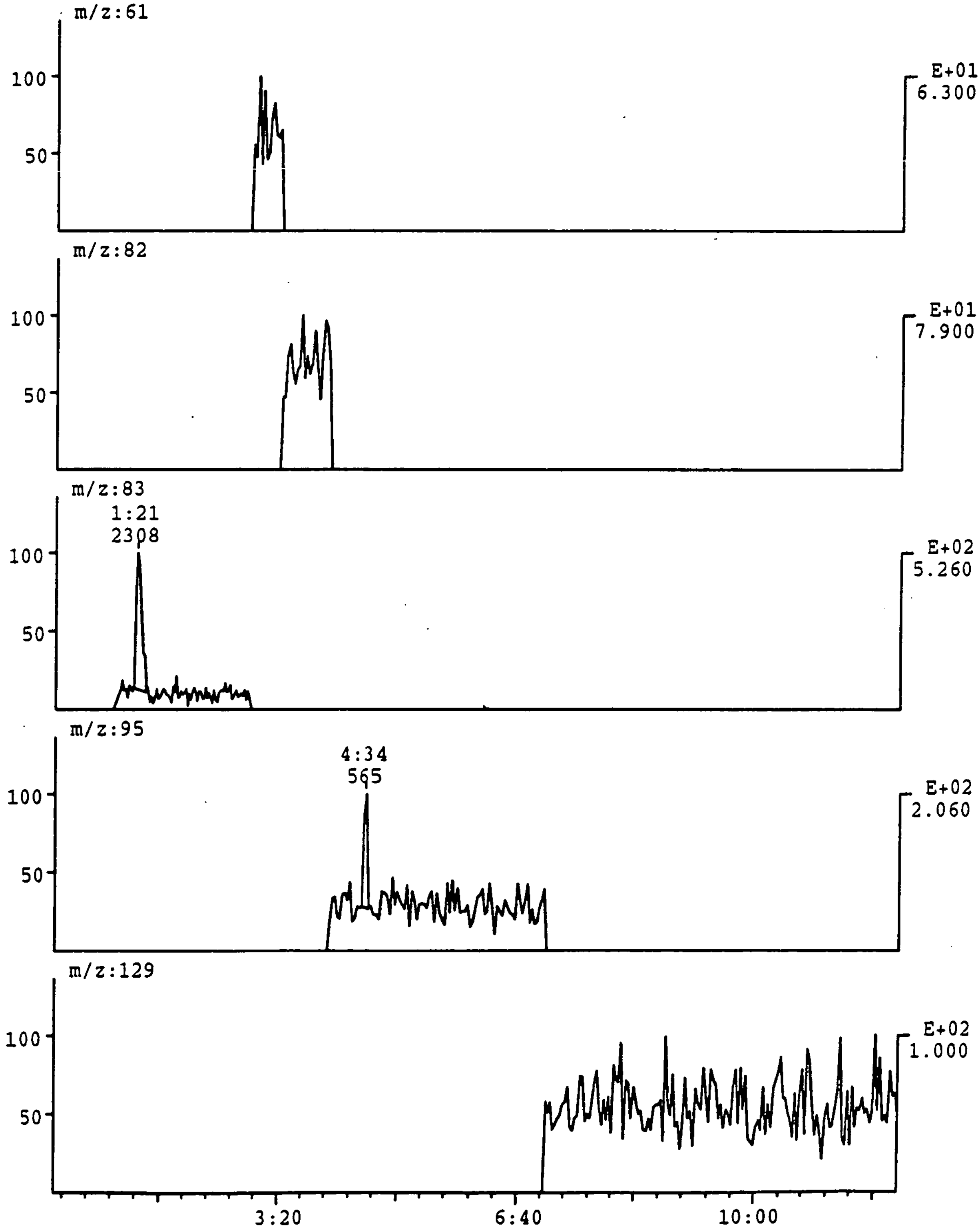


```
CHRO:  rba06                                  04-MAY-94  Elapse:  00:06:37.1   221
Samp:  devilla 210494 veg blank                          Start :  14:35:57     377
Comm:  SE- 54 30m 0.32mmid 1um film he 7psi  seq no 2 022/004
Mode:  EI +DAU LMR GAS UP LR                             Study :  Halocarbons
Oper:  A.Newton     Client:  Ruth Brown                  Inlet :  GC      Vial   6
Peak:  1000.00  mmu    Label wndw:   1 > 377             Masses:  61 > 129
Area:  3, 3.00         Baseline  :   0, 3                Label :  2, 5.00
Disp:  Area
```

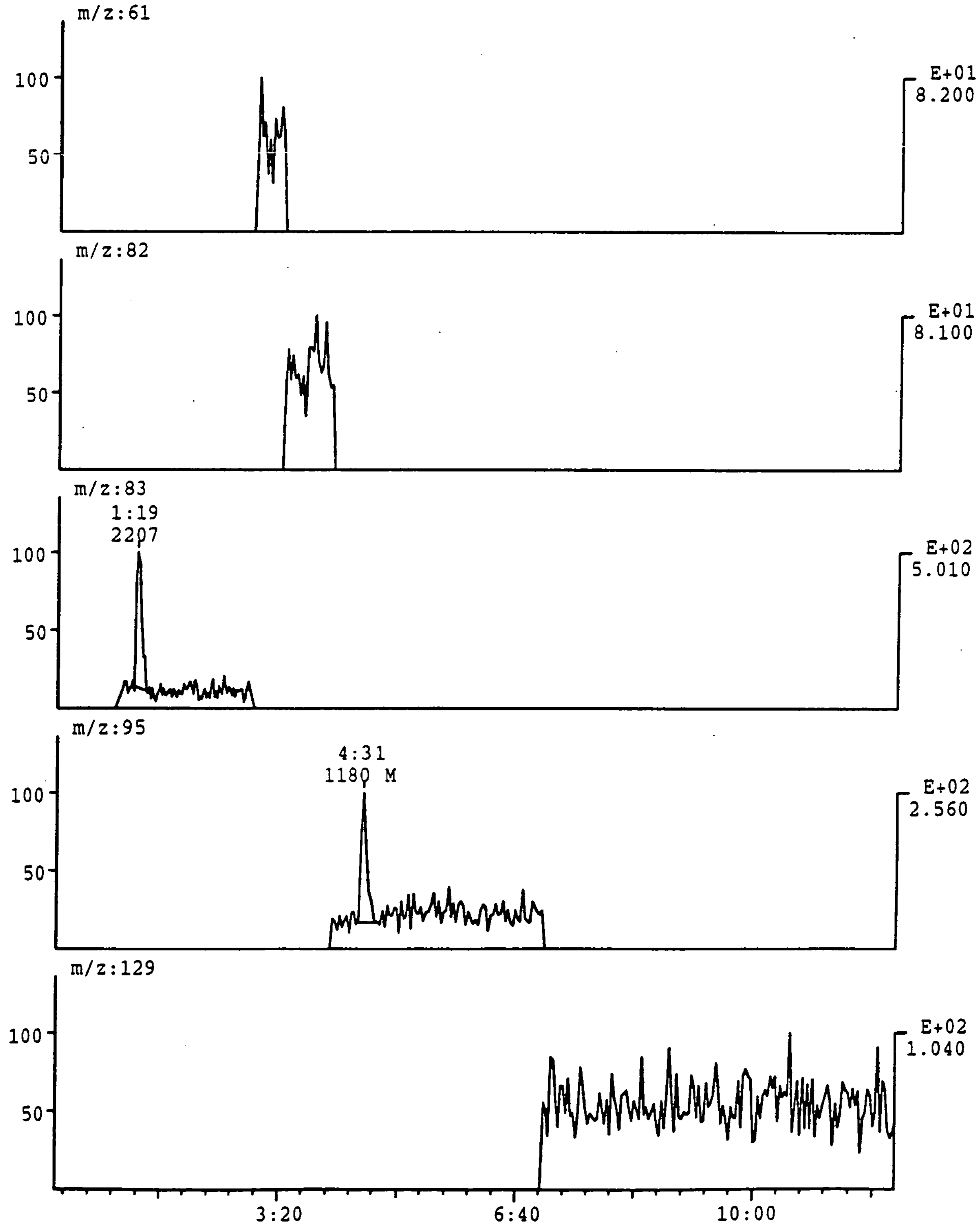


se note 6.

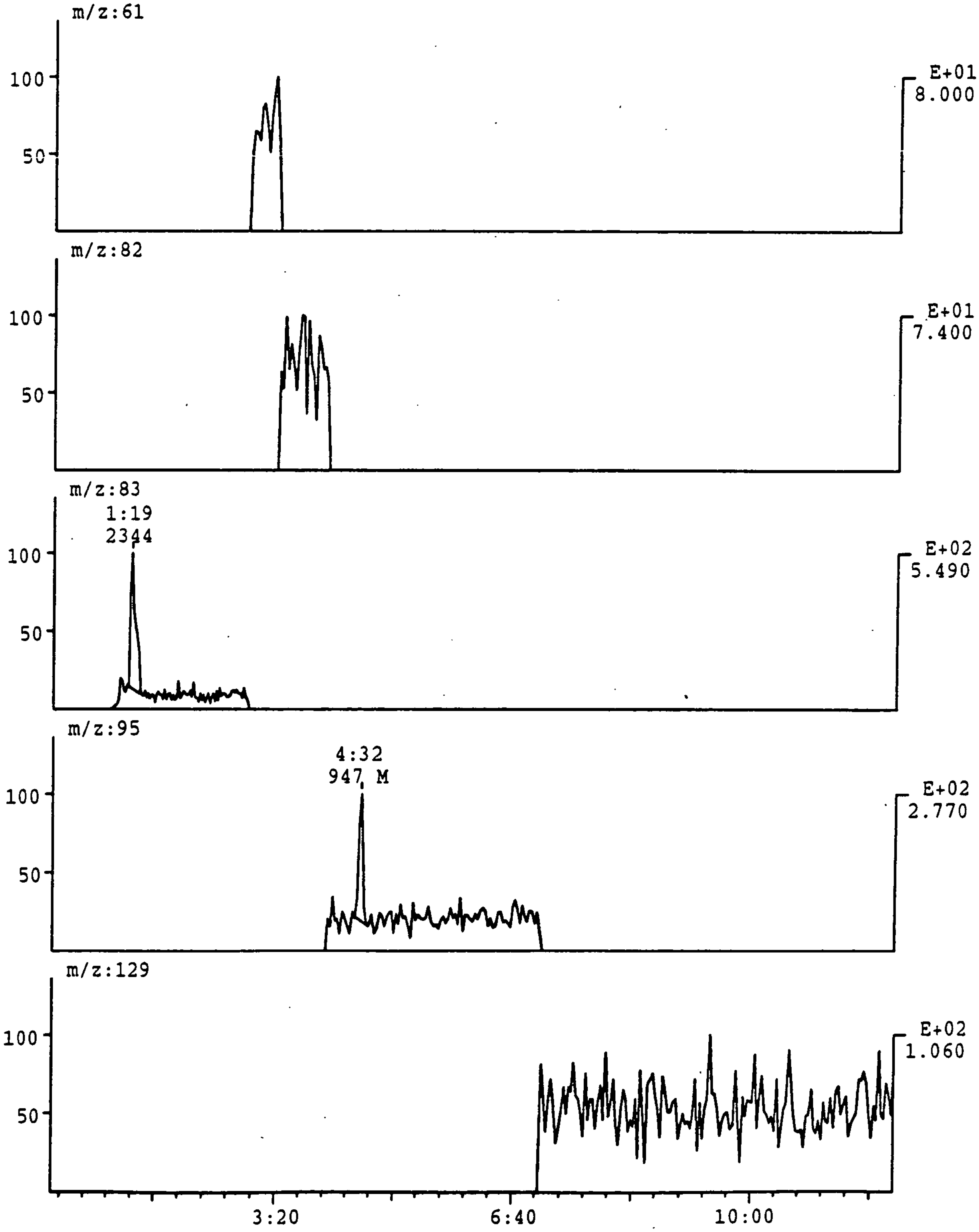
```
CHRO:  rba07                               04-MAY-94  Elapse:  00:06:37.0   221
Samp:  devilla 210494 veg 40 1992                     Start :  14:53:13     377
Comm:  SE- 54 30m 0.32mmid 1um film he 7psi  seq no 2 022/004
Mode:  EI +DAU LMR GAS UP LR                          Study :  Halocarbons
Oper:  A.Newton      Client:  Ruth Brown              Inlet :  GC      Vial   7
Peak:  1000.00  mmu     Label wndw:   1 > 377         Masses:  61 > 129
Area:  3, 3.00          Baseline  :   0, 3            Label :  2, 5.00
Disp:  Area
```



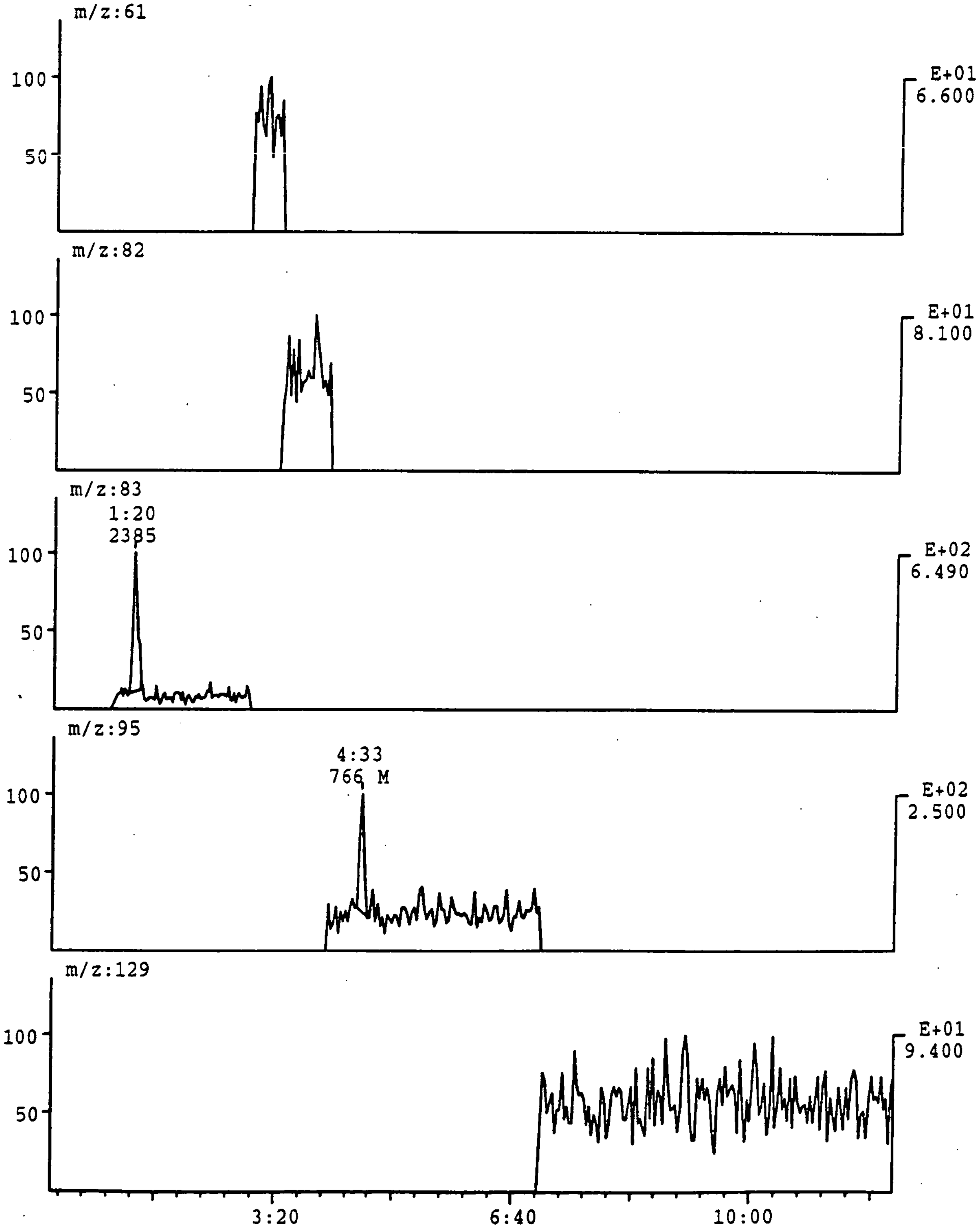
```
CHRO:  rba08                             04-MAY-94  Elapse:  00:06:36.0   221
Samp:  devilla 210494 veg 40 1993                   Start :  15:26:25     377
Comm:  SE- 54 30m 0.32mmid 1um film he 7psi  seq no 2 022/004
Mode:  EI +DAU LMR GAS UP LR                        Study :  Halocarbons
Oper:  A.Newton      Client:  Ruth Brown            Inlet :  GC      Vial   8
Peak:  1000.00  mmu    Label wndw:   1 > 377        Masses:  61 > 129
Area:  3, 3.00         Baseline  :   0, 3           Label :  2, 5.00
Disp:  Area
```



```
CHRO:  rba09                                 04-MAY-94  Elapse:  00:06:37.1   221
Samp:  devilla 210494 veg 30 1992                       Start :  15:59:45     377
Comm:  SE- 54 30m 0.32mmid 1um film he 7psi  seq no 2 022/004
Mode:  EI +DAU LMR GAS UP LR                            Study :  Halocarbons
Oper:  A.Newton      Client:  Ruth Brown                Inlet :  GC      Vial   9
Peak:  1000.00  mmu    Label wndw:   1 > 377            Masses:  61 > 129
Area:  3, 3.00         Baseline  :   0, 3               Label :  2, 5.00
Disp:  Area
```



```
CHRO:  rba10                                  04-MAY-94  Elapse:  00:06:35.9   221
Samp:  devilla 210494 veg 30 1993                        Start :  16:33:24     377
Comm:  SE- 54 30m 0.32mmid 1um film he 7psi  seq no 2 022/004
Mode:  EI +DAU LMR GAS UP LR                             Study :  Halocarbons
Oper:  A.Newton       Client:  Ruth Brown                Inlet :  GC      Vial  10
Peak:  1000.00  mmu     Label wndw:   1 > 377            Masses:  61 > 129
Area:  3, 3.00          Baseline  :   0, 3               Label :  2, 5.00
Disp:  Area
```



Peak area and retention time (t_R in mins) data for the six calibration sets analysed during the autosampler run. CH_3I and $CHCl_3$ data are excluded due to artefact formation. Mean and standard deviation values for t_R are in bold.

Chlorinated solvent	**N**	**Calibration standard concentration (μg/ml)**					
		0.025		**0. 05**		**0. 5**	
		t_R	peak area	t_R	peak area	t_R	peak area
$C_2H_3Cl_3$	**1**	3.09	825	3.11	1794	3.09	17952
	2	3.11	715	3.07	1710	3.09	16146
	3	3.11	641	3.08	1587	3.11	13666
	4	3.09	715	3.09	1215	3.07	11216
	5	3.07	814	3.07	1132	3.07	10973
	6	3.09	433	3.07	908	3.07	9432
		3.09		**3.08**		**3.08**	
		±0.01		**±0.02**		**±0.02**	
CCl_4	**1**	3.32	2120	3.34	3569	3.30	34781
	2	3.34	1739	3.29	2655	3.32	32344
	3	3.32	1489	3.29	2627	3.32	26092
	4	3.30	1450	3.30	2377	3.30	23963
	5	3.30	1133	3.29	2583	3.30	21256
	6	3.32	1209	3.29	2085	3.30	19924
		3.22		**3.30**		**3.31**	
		±0.01		**±0.02**		**±0.01**	
C_2HCl_3	**1**	4.32	3023	4.34	6539	4.30	60382
	2	4.34	2003	4.30	4706	4.32	49673
	3	4.32	2145	4.29	3511	4.32	36945
	4	4.30	1652	4.30	3447	4.30	31986
	5	4.30	2045	4.30	3230	4.30	30867
	6	4.32	1445	4.30	2364	4.30	28146
		4.32		**4.31**		**4.31**	
		±0.01		**±0.02**		**±0.01**	
C_2Cl_4	**1**	10.45	5481	10.43	10791	10.43	108459
	2	10.45	4410	10.40	9089	10.41	103942
	3	10.41	3106	10.37	7444	10.43	77201
	4	10.36	2894	10.36	5779	10.36	62372
	5	10.36	3103	10.36	5638	10.39	57556
	6	10.39	2360	10.36	4491	10.36	51415
		10.40		**10.38**		**10.40**	
		±0.04		**±0.03**		**±0.03**	

GC/MSMS appendix		**Calculation for concentrations found in Devilla needles**						
m/z=95 (C2HCl3)		**sampled on 21/04/94**						
				n=3				
standard ug/ml	**peak area**			Standard error of y on x				361
0.025	3023			Standard deviation of y on x = s.e.*sqrt(n)				625
0.05	6539			limit of detection = intercept + 3* s.d.				2138
0.5	60382			**equivalent concentration for LOD**				**0.0156 ug/ml**
			field-blank corrected					
Needle samples	**peak area**	**calculated ug/ml**	**ng/g dry weight**					
Devilla field blank	588	0.0049						
Devilla 13m 1992	565	0.0047	-0.81					
Devilla 13m 1993	1180	0.0098	20					
Devilla 10m 1992	947	0.0078	12					
Devilla 10m 1993	766	0.0063	5.7					
		NB all <LOD						

Regression Statistics	for	Calibration				
Multiple R	0.999968546					
R Square	0.999937092					
Adjusted R Square	0.999874184					
Standard Error	360.6117757					
Observations	3					
Analysis of Variance						
	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	
Regression	1	2067031888	2067031888	15895.25	0.005049	
Residual	1	130040.8528	130040.8528			
Total	2	2067161929				
	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	264.2521866	277.0801143	0.953703182	0.440886	-3256.37	3784.874
x1	120263.0321	953.8903719	126.0763664	6.29E-05	108142.8	132383.3

Appendix 8 : Example calculation for the bioconcentration factor (BCF)

Partition ratio data ($g.m^{-3}/g.m^{-3}$)

The standard atmosphere data (g/m^3,Chapter 4) is from the 0.5 L/min N_2 flow with mass loss, for example :

$$\frac{(12.76-11.73)\ g}{23\ \text{hours}\ 10\ \text{mins}} = \frac{1.027}{1390} = 0.000739\ g/min \text{ which is } 1.477 g/m^3 \text{ at } 0.5 L/min$$

The needle data for the partition ratios is converted from the ng/g fresh weight data obtained by experiment to $g_{\text{fresh weight}}/m^3$ by dividing by $\rho_{\text{fresh weight}} = 0.89\ g/cm^3$ (Appendix 6) and 1000 (mg/m^3 to g/m^3).

The wax data (g/m^3) for the partition ratios was obtained from the concentration of chlorocarbon in the wax (ng/ml pentane) and the mass of wax coating the disc. The density of wax was measured as $0.82 g/cm^3$ (Appendix 2), for example :

concentration of chlorocarbon in wax extract (corrected for the blank) = 374 ng/ml
mass of wax on disc = 1.17mg
extraction volume of pentane = 1ml

As all the wax was extracted by the pentane the concentration of solvent (g/m^3)

$$= \left[\frac{374ng}{1.17mg}\right] \text{ x } 0.82\text{x}10^6 (g/m^3) = 262 g/m^3$$

Bioconcentration factor data ($g.g^{-1}/g.g^{-1}$)

The bioconcentration factor is the air : needle (or wax) partition data expressed as g chlorocarbon.$g^{-1}_{\text{fresh needle (wax)}}$/g chlorocarbon.$g^{-1}_{\text{air}}$.
The partition ratio ($g.m^{-3}/g.m^{-3}$) data for $C_2H_3Cl_3$ used for the BCF calculation are in the tables below:

Table 1 Needle data for partition ratio and BCF calculations

Standard atmosphere concentration (g/m^3)	Standard atmosphere concentration (µg/g)	Concentration in ng/g $_{\text{fresh weight of needles}}$	Concentration in g/m^3 $_{\text{fresh weight of needles}}$
0.0024	2.0	15	0.017
0.0071	5.9	35	0.039
0.43	360	5840	6.5
0.52	430	7400	8.2
1.5	1230	17300	19

Conversion of standard atmosphere data from g/m^3 to µg/g for the BCF calculation uses air density as 1.19g/L (Bacci *et al.*, 1990).

The concentration of the solvent in the wax (g/g) was also obtained from the experimental concentration of chlorocarbon in the wax (ng/ml pentane) and the mass of wax coating the disc. Using the example above:

$$\text{Concentration of solvent in the wax (g/g)} = \frac{374ng}{1.17mg} = 319.7\mu g/g$$

Table 2 Wax data for BCF calculations

Standard atmosphere concentration (g/m^3)	Standard atmosphere concentration (μg/g)	Concentration in $\mu g/g_{wax}$
0.011	9.3	10
0.072	60	23
		25
		32
		39
0.31	260	206
		210
0.53	440	320

Bioconcentration factor calculation

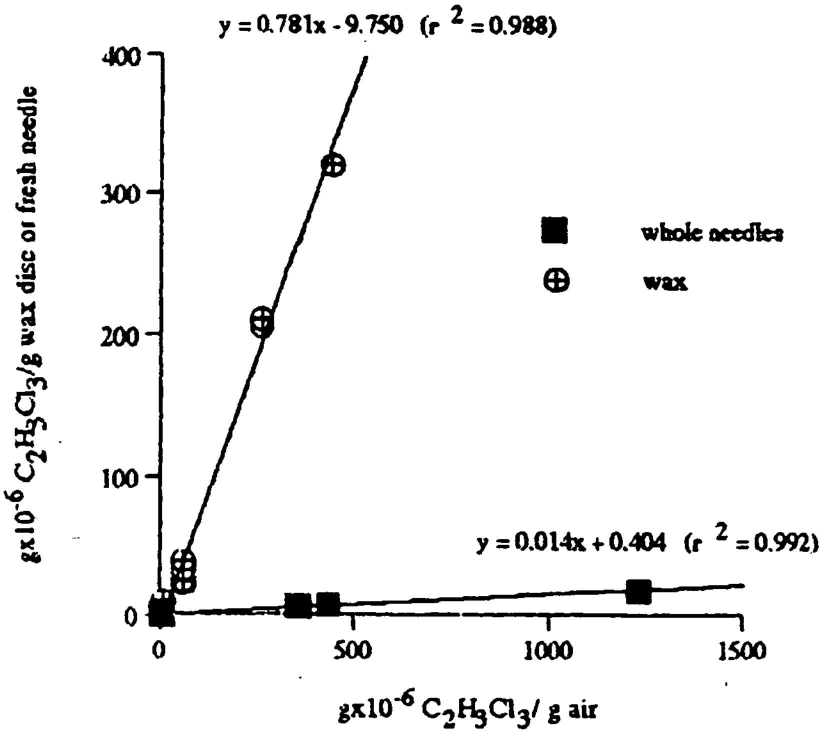
The air and needle (or wax) concentrations in μg/g were plotted as below, the slope of the regression line gives the $BCF_{\text{fresh weight needle}}$ and BCF_{wax} respectively.

$$BCF_{wax} = 0.781$$
$$BCF_{\text{fresh weight needle}} = 0.014$$

If all $C_2H_3Cl_3$ is adsorbed in the wax, then $g_{wax}/g_{\text{fresh weight needle}}$

$$= \frac{0.014}{0.781} = 2\%$$

In practice, this value is nearer 1% (Cape, 1986), thus half the solvent is in the wax and half is in the rest of the needle.



APPENDIX 9 Complete data set

ISR value and internal standard peak areas for all needle, wax and calibration data

Sampling dates are in bold underneath the analysis date

Artefacts are due to chromatographic abnormalities (Chapters 2 and 3)

ISR values for CHCl3 were not calculated for the wax data owing to contamination (Chapter 5)

Needle and air sampler data

Analysis Date	Sample description	Run no.	Internal standard peak area	CHCl3 ISR	C2H3Cl3 ISR	CCl4 ISR	C2HCl3 ISR	C2Cl4 ISR
10/06/93	bl	104	63876028	0.00896	0.00152	0.00973	0.00471	0.00023
01/04/93	bl	105	56742464	0.0086	0.00118	0.0224	0.0174	0.00158
	bl	106	45223897	0.00668	0.00009	0.00567	0.0148	artefact
	ponw92	108	54772108	0.0107	0.00343	0.00483	0.0217	0
	ponw92	109	49066848	0.00487	0.00084	0.00564	0.0259	artefact
	ponw92	110	35334360	0.024	0.00569	0.00666	0.0283	0.00222
22/06/93	bl	121	66217768	0.00276	0.00045	0.00428	0.00455	0.00223
25/03/93	bl	122	51720744	0.00239	0.00097	0.00896	0.0141	0.00666
	bl	123	59099096	0.00297	artefact	0.00657	0.00567	0.00733
	ban92	124	32799804	0.00268	artefact	0.00811	0.0291	0.0138
	ban92	125	45889800	0.0438	0.00469	0.00648	0.0128	artefact
	ban92	126	40414276	0.0235	0.00247	0.00664	0.0194	0.00921
24/06/93	bl	131	67170008	0.0256	0.00028	0.00435	0.00991	0.00045
15/03/93	bl	132	49789212	0.0139	0.000912	0.00779	artefact	0.00025
	bl	133	30281988	0.0168	0	0.0125	0.00155	artefact
	dev13m92	134	47207552	0.0588	0.00272	0.00834	0.0249	0.0038
	dev13m92	135	35238632	0.0649	0.0121	0.0107	0.0349	0.00154
	dev13m92	136	25718936	0.0494	0.0195	0.015	artefact	0.00477
	dev10m92	137	56969940	0.0578	0	0.0154	0.0216	0.00128
	dev10m92	138	38428932	0.0244	0.0324	0.0078	0.0343	artefact
	dev10m92	139	36402820	0.0532	0	0.0185	0.0319	0.00395
25/06/93	bl	158	45150388	0.0599	0.00362	0.00714	0.0172	0.00055
25/06/93	bl	159	49336880	0.0244	0	0.00932	0.025	artefact
	ponw92	160	37456072	0.0437	0	0	0.0229	0.00427
	ponw92	161	32943446	0.0545	0	0.00171	0.0178	0
	ponw92	162	38869304	0.0566	0	0.00433	artefact	artefact
31/05/93	bl	163	39877709	not calc	0.00218	0	0.00116	0
	bl	164	37612233	not calc	0.00547	0.00334	artefact	0
	bl	165	40121145	not calc	0.00479	0.00124	0.00658	0
	ban1air	166	28097798	not calc	0.0325	0.00113	0.00769	0.00039
	ban1air	167	32510954	not calc	0.00885	0	0.00582	0.0004
	ban1air	168	33151812	artefact	0	artefact	0	
29/06/93	bl	170	35243824	0.0622	0.00341	0.0124	0.00617	0
31/05/93	bl	171	28069728	0.085	0.009	0.0185	0.0379	0.0126
	bl	172	33857320	0.0861	0	0.00915	0.00722	0
	bl	173	34658028	0.1033	artefact	0.00642	0.00433	0
	ban92	174	39988248	0.0903	0.00507	0.00539	0.025	0.0104
	ban92	175	38164312	0.0594	artefact	0.00501	0.0276	0.012
	ban92	176	50957376	0.07318	0.00453	0.00385	0.0227	0.0005
01/04/93	ponc92	177	22313162	0.125	0.00083	0.0029	0.03	0.00914
	ponc92	178	17717858	0.092	artefact	0.00345	0.0538	artefact
	ponc92	179	26825728	0.108	0	0.00617	artefact	0.00492
30/06/93	ban2air	181	23651876	not calc	artefact	0.0008	artefact	0.006

31/05/93	ban2air	182	24242110	not calc	0.00444	0	0.00707	0
	ban2air	182	30001244	not calc	0.0544	artefact	0.00554	0
	ban3air	183	26751209	not calc	0.00611	0	0.00652	0
	ban3air	184	26755413	not calc	0.0676	0.0009	0.00824	0
	ban3air	185	28876199	not calc	artefact	0	0.00899	0
09/07/93	bl	186	31558345	not calc	0	0	0.00005	0
	bl	187	28109087	not calc	0	0.0052	0	0
	bl	188	26709903	not calc	0	0	0.00924	0
	ban1air	190	21542765	not calc	0.0023	0	0	0
	ban1air	191	28027186	not calc	0.0011	0.0053	0	0.0059
	ban1air	192	27196663	not calc	0	0.00426	0.00705	0
	ban2air	193	23232410	not calc	0	0	0	0
	ban2air	194	31983487	not calc	0.00245	0.0011	0.00687	0
	ban2air	195	29036587	not calc	0	0.0032	0.00314	0
	ban3air	196	41171138	not calc	0.00387	0.00673	0.00778	0
	ban3air	197	45298567	not calc	0.00477	0.00498	0	0
01/07/93	bl	199	71107584	0.14	0.00512	0.00339	0.001	0.00234
11/06/93	bl	200	42304888	0.119	0.00283	0.00963	0.00197	artefact
	bl	201	34025504	0.191	0.00633	0.00285	0.0165	0.0215
	dev10m92	202	21380576	0.18	0.000438	0.0127	0.0209	0.00249
	dev10m92	203	30379792	0.145	0.00982	0.00264	0.0106	0.00062
	dev10m92	204	39698212	0.108	0	0.00743	0.0491	artefact
	dev13m92	205	33928592	0.0818	0	0.00295	0.00737	artefact
	dev13m92	206	34593108	0.0572	artefact	0.00683	0.0261	0.0147
	dev13m92	207	29166064	0.124	0.00257	0.00773	0.0365	0.0136
01/04/93	bl	208	25681987	not calc	0.00025	0.00572	0.021	0.00309
	bl	209	23164434	not calc	0.00309	0.00667	0.0283	0.0024
	bl	210	20009132	not calc	0	0.00558	0.0236	0
	ponswair	211	28610923	not calc	0	0.00265	0.0297	0.0044
	ponswair	212	25511328	not calc	artefact	0.00093	0.0343	0.00127
	ponswair	213	24186534	not calc	0.0075	0.00533	0.0255	0
22/11/93	ban92a	241	21530374	1.53	0.0497	0.0706	0.00147	0.00149
04/11/93	ban92a	242	14995311	1.51	0.0457	0.0506	0.0119	0.016
	ban92a	243	14244210	1.68	0	0.0696	0.00956	0.0229
24/11/93	fieldbl	249	15150162	1.69	0.0671	0.0367	0.00306	0.00113
	fieldbl	250	14580508	1.81	0	0.101	0.0081	8.8E-05
	ban93a	253	25169760	1.69	0.0017	0.0493	0.00575	0.0123
	ban93a	254	15724261	1.68	0.0122	0.0899	0.00862	0.0167
	ban93a	255	16288359	0.959	0	0.0893	0.00842	0.021
25/11/93	ban3,92	257	29031494	1.05	0.052	0.0418	0.0037	0.00845
	ban3,92	258	17070988	1.75	0	0.0515	0.00779	0.0172
	ban3,92	259	18493310	1.97	0	0.0748	0.00746	0.0157
	ban2,92	260	19024268	1.65	0.0732	0.0643	0.00422	0.0143
	ban2,92	261	18375324	2.33	artefact	0.0679	0.00409	0.0129
	ban2,92	262	19059636	2.08	0	0.0708	0.00433	0.0129
30/11/93	ban1,92	265	15191504	2.94	artefact	artefact	0.0136	0.0233
	ban1,92	266	11994538	2.33	0	0.0652	0.0105	0.0196
	ban1,92	267	24695026	2.69	0.0455	0.0824	0.0134	0.0242
01/12/93	ban3,93	270	32028620	2.06	0	0.0505	0.0135	0.0143
	ban3,93	271	15835301	2.41	0	0.0972	0.00326	0.0268
	ban3,93	272	13223606	3.28	0.0098	0.0369	artefact	0.00898
	ban2,93	273	27105992	1.975	0.0123	0.0521	0.015	0.0199
	ban2,93	274	28944890	3.333	0.0269	0.0809	0.0189	0.0276
	ban2,93	278	33892003	2.797	0	0.0616	0.00699	0.0107
02/12/93	ban92froz	279	21523930	3.73	0	0	0.00921	artefact
	ban92froz	280	24821138	2.76	0.0551	artefact	0.00444	0.00144
	ban92froz	281	41449148	2.86	artefact	0.0699	0.00246	0.0179

	ban93froz	282	22373028	2.61	0.019	0.083	0.00687	0.01286
	lab bl	283	41285268	2.32	0.347	0.0696	0.01921	0.0171
	ban93froz	284	44908412	2.94	0.00149	artefact	0.00881	0.0112
	ban93froz	285	11532713	2.77	0	0	artefact	0
03/12/93	lab bl	286	28946663	6.44	0.187	0.0671	0.0189	0.018
	lab bl	287	22243056	4.54	artefact	0.0867	0.012	0.0103
	ban1,93	288	21686106	3.672	0	0.067	0.0106	0.0116
	ban1,93	289	16713511	2.784	0	0.0806	0.021	0.0206
	ban1,93	290	21984463	4.175	0.0038	0.0909	artefact	artefact
01/04/93	ponscair	291	32198336	not calc	0	0	0.0537	0.00351
	ponscair	292	29091742	not calc	0	0	0.0188	artefact
	ponscair	293	29018367	not calc	0	0.00402	0.0418	0.0028
	ponseair	294	32673823	not calc	0	0.0104	0.0253	0
	ponseair	295	34527289	not calc	0	0	0.0214	0
	ponseair	296	36737282	not calc	0.0027	0.00261	0.0237	0.00306
05/05/93	bl	297	36109833	not calc	0	0.00325	0.00017	0.00449
	bl	298	35626728	not calc	0	0	0.0275	0.0113
	bl	299	20384673	not calc	0	0	artefact	0
	dev13air	300	19273647	not calc	0.0044	0.00838	0.013	0
	dev13air	301	21374637	not calc	0.00537	0	0.02	0.00633
	dev13air	302	22746239	not calc	artefact	artefact	artefact	0
	dev10air	303	28765367	not calc	0	0.01	0.021	0
	dev10air	304	18883654	not calc	0.0135	0.0065	0.0113	0.00388
	dev10air	305	20043863	not calc	0.00568	0	artefact	0
	dev7air	306	39827636	not calc	0.00894	0.0068	0.0108	0.0076
	dev7air	307	40293745	not calc	0.00313	0	0.0148	0
	dev7air	308	27438495	not calc	0.00357	0	artefact	0
	ban92frid	309	27664028	4.31	0.0329	0.051	0.0073	0.007
	ban92frid	310	30292996	4.29	0.0421	0.0327	0.0037	0.0188
	ban92frid	311	27140778	4.99	0.0169	0.0426	0.00991	0.0112
13/01/94	bl	326	18074696	0.0781	0	0	0.0111	0.00307
14/01/94	bl	328	42862904	0.323	0.000261	0	0.0158	0.00158
11/08/93	dev13m92	329	15572843	0.152	0	0	0.0236	0.00151
	dev13m92	330	17549602	0.194	0.0205	0	0.0163	artefact
	dev13m92	331	13740954	0.24	0	0	0.0227	0.00259
	bl	332	10212282	0.757	0	0	0.0257	9.2E-05
	dev13m93	333	27911456	0.322	0	0	0.0155	0.00288
	dev13m93	334	13262645	0.184	0	0	0.0252	0.00388
	dev10m92	338	15259687	0.357	0.00718	0	0.0197	0.0009
17/01/94	dev10m93	339	22312136	0.114	0.0105	artefact	0.00459	0
	dev10m93	340	30678522	0.117	0.00822	0	0.0152	0.00863
	dev10m93	341	23542664	0.328	0.0093	0	0.0233	0.00157
05/07/93	bl	342	46327848	not calc	0.00186	0.00324	0.0193	0.00121
	bl	343	47383940	not calc	0.00166	0.00277	0.0197	0
	bl	344	36374859	not calc	0.00191	0.00338	0.019	0
	ponswair	345	43948756	not calc	0.00093	0	0.0173	0
	ponswair	346	49394757	not calc	0	0.00329	0.018	0
	ponswair	347	29475849	not calc	0.00276	0.00298	0.0218	0.00289
	ponscair	348	48404856	not calc	0	0.00315	0.0119	0
	ponscair	349	29347469	not calc	0.00147	0.00334	0.0222	0
	ponscair	350	29384765	not calc	0	0.0039	0.0231	0.0042
	ponseair	351	40003943	not calc	0	0	0.00932	0
	ponseair	352	38484903	not calc	0	0	artefact	0
	ponseair	353	39438547	not calc	0	0	0.024	0
24/01/94	bl	355	49038349	not calc	0	0	0	0.0519
11/08/93	bl	356	30293847	not calc	0	0	0	0.0657
	bl	357	20938947	not calc	0	0.00037	0.0023	0.0325

	dev13air	358	39383746	not calc	0.0303	0.0006	0	0.0574
	dev13air	359	38273647	not calc	0.066	0.0012	0.0103	0.1066
	dev13air	360	39238375	not calc	artefact	0	0.00623	0
	dev10air	361	47888593	not calc	0.009	0.0002	0	0.0533
	dev10air	362	37666465	not calc	0.0447	0.0003	0.00245	0.0413
	dev10air	363	23903846	not calc	0.056	0	0.0021	0.0596
	dev7air	364	36453637	not calc	0.032	0	0	0.0371
	dev7air	365	39484655	not calc	artefact	0	0.0068	0.0587
	dev7air	366	39348755	not calc	0	0	0	0.0562
25/01/94	bl	368	29983645	not calc	0	0.00476	0.0222	0
23/09/93	bl	369	20002865	not calc	0.00486	0.00399	0.0035	0
	bl	370	20473654	not calc	0.00483	0.00393	0.01	0.0104
	ponswair	371	23847467	not calc	0	0	0.0212	0.0047
	ponswair	372	39484575	not calc	0	0.0126	artefact	0.00665
	ponswair	373	34634533	not calc	0	0	0.0197	0
	ponscair	374	20947846	not calc	0.0931	0.0157	0.0333	artefact
	ponscair	375	35365473	not calc	0.0867	0.0192	artefact	0.00286
	ponseair	376	20494756	not calc	0	0.0079	0.023	0.0105
	ponseair	377	25349566	not calc	0.021	0.00629	0.019	0.00336
26/01/94	bl	401	35333720	0.0231	0.00225	artefact	0.00975	0
24/01/94	bl	402	54016484	0.00997	0.00246	0.00316	0.00196	0.00118
	ban93	403	36872768	0.0197	0.00386	0	0.00149	0.00073
	ban93	404	32507988	0.0751	0.0022	0	0.00827	0.00084
	ban93	405	25938854	0.1455	0.00651	0	0.00698	artefact
27/01/94	ban92	407	43061680	0.861	0.0053	0	0.0122	0.00293
	ban92	408	36018024	1.407	0.00967	0.00571	0.0221	artefact
	ban92	409	22451252	1.167	0.00638	artefact	0.00981	0
11/02/94	bl	414	36047304	1.776	0.00331	0.0758	0.0161	0.0148
23/11/93	bl	415	12220608	2.038	0.00251	0.0204	0.00279	0.0105
	bl	416	19937482	1.939	0.0132	0.099	0.0114	0.0175
14/02/94	dev10m92	419	10800929	2.056	0.0419	0.0149	0.0385	0.0171
	dev10m92	420	15012074	2.006	0.00301	0.0813	artefact	0.100596
	dev10m92	421	10760568	1.915	none	0	0.0228	none
	dev13m92	422	13372716	2.223	0.00385	0.0924	0.0229	0.0221
	dev13m92	423	13056981	2.217	0.00446	0.0834	0.0262	0.0242
	dev13m92	424	11456433	2.99	0.00574	0.0809	0.0384	0.03
	dev13m93	425	58476393	2.812	0.00492	0.0912	0.0307	0.0245
	dev13m93	426	14269106	1.796	0.00395	0.063	0.0283	0.0216
	dev13m93	427	14037332	1.93	artefact	0.0722	0.0336	0.0251
15/02/94	dev10m93	429	14909090	1.904	0.00382	0.0684	0.0194	0.0151
	dev10m93	430	17423146	2.005	0.00329	0.0393	0.00555	0.0104
	dev10m93	431	15939314	1.963	0.00409	0.0872	0.0207	0.0211
17/02/94	bl	433	15146867	0.00708	0.000866	0.00243	0.009	0.0092
16/02/94	bl	434	15998166	0.0168	0	0.0146	0.03	0.00675
18/02/94	bl	441	16412216	0.0102	0.00683	0.00372	0.0151	0
	ponsw93	442	15267335	0.0426	0.00517	0.0064	0.0337	artefact
	ponsw93	443	17224440	0.0452	0.00275	0.00737	0.0176	0.0148
	ponsw93	444	14049732	0.06	0.00838	0.00847	0.0349	0.00602
	ponsw92	445	10613214	0.0477	0.00707	0.0183	0.0187	0.00332
	ponsw92	446	11847648	0.0706	0.00436	0.0119	0.0375	0.0192
	ponsw92	447	37947092	0.0722	0.00446	0.0116	0.0476	0.00606
	ponsc92	448	57128614	0.0567	0.00195	0.00341	0.00427	0.00907
	ponsc92	449	13245170	0.123	0.0104	artefact	0.0626	0.0182
21/02/94	ponsc92	451	38131592	0.0724	0.0125	0.0253	0.0271	0.0137
	ponsc93	452	30760906	0.165	0.00843	0.0122	0.0376	0.0127
	ponsc93	453	16278220	0.358	0.0135	0.0314	0.0184	0.02
24/01/94	bl	454	18836734	not calc	0.0044	0.00582	0.00789	0.00746

	bl	455	38474639	not calc	0.0068	0.0108	0.013	0
	bl	456	28846473	not calc	0.00711	artefact	0.00695	0.00253
22/02/94	ponsc93	458	14396684	0.174	0.0128	0.0499	0.0783	0.00367
	ponse92	460	15074421	0.379	0.00895	0.00953	0.0365	0.0113
	ponse92	461	10859980	0.421	0.00487	0.0115	0.0233	0.0105
	ponse92	462	13598354	0.365	0.00527	0.0118	0.0439	artefact
23/02/94	ponse93	466	41108643	0.043	0.0055	0.0156	0.0062	0.0113
	ponse93	468	19046385	0.0539	artefact	artefact	0.0469	artefact
	ponse93	469	43442604	0.268	0.00705	0.0186	0.039	0.0105
	ban1air	470	36484948	not calc	0.0411	0.0034	0.0164	0.00446
	ban1air	471	23947846	not calc	artefact	0.00922	0.00839	0.00624
	ban1air	472	48474748	not calc	0.0098	0.009	artefact	0.00555
	ban2air	473	26787467	not calc	0.0717	0	0.00998	0
	ban2air	474	30980074	not calc	0.00666	0.018	0.0113	0.0077
	ban3air	475	31036213	not calc	0.0116	0.0134	0.0212	0
	ban3air	476	21128373	not calc	0.0069	0.00901	0.0148	0.0087
02/03/94	bl	480	17930314	0.289	0.00261	0	0.0111	0.00307
	bl	481	20160054	0.00027	0	0	0.0158	0.00158
	bl	482	78293536	0.00504	0	0	0.0257	9.2E-05
03/03/94	ponw92	489	12383296	0.138	0	0	0.0319	artefact
23/09/93	ponw92	491	22121117	0.0946	0	0	0.0118	0.00215
	ponw92	492	29520428	0.0417	0	artefact	0.0296	0
	ponc92	493	21088516	0.223	0.00158	0	0.0276	0
04/03/94	ponc92	496	39285875	0.324	0	0.0286	0.0247	0.0113
	ponc92	497	53878423	1.173	0.0257	artefact	0.0469	0.019
	pone92	498	21372629	0.103	0	0	0.0334	0.0027
	pone92	499	49244613	0.148	0	0	artefact	0.00354
	pone92	501	58243962	0.302	0.00474	artefact	0.0322	0.00335
07/03/94	dev10m93	505	47761114	0.458	0.00178	0.00757	0.0331	0.00053
10/02/94	dev10m93	506	83208121	0.457	0	0.00804	0.0243	0.00424
	dev10m93	507	11126742	0.995	artefact	0.00723	0.0258	0.00197
08/03/94	dev13m93	510	17680288	0.772	0.00937	0.0171	0.0193	0.00803
	dev13m93	511	70241956	0.727	0.00483	0.00834	0.0321	0.00241
	dev13m93	512	35538718	0.555	0.00274	artefact	0.0488	0.00287
	dev10m92	514	62323600	0.487	0.00836	0.0152	0.0284	0.00303
	dev10m92	516	12966738	0.508	0.00698	0.0106	0.0177	0.00314
	dev10m92	517	80000856	0.446	0.00946	0.0167	0.04	0.00326
09/03/94	dev13m92	520	76663480	0.486	0.00497	0.00687	0.0302	0.00224
	dev13m92	521	10443555	0.53	0.00831	0.00371	0.0366	0.00203
	dev13m92	522	11008240	0.455	0.0402	0.0121	0.0345	0.0047
23/11/93	bl	566	29348548	not calc	0.0503	0	0.0228	0
	bl	567	22283763	not calc	0.0683	0.00485	0.00999	0
	bl	568	21092776	not calc	0.0626	artefact	0.0146	0
	dev13air	569	29838737	not calc	0.0686	0	0.0255	artefact
	dev13air	570	39282373	not calc	0.0685	0.0132	0.0178	0
	dev13air	571	30002011	not calc	0.0579	0.0104	0.00922	0.00389
	dev10air	572	39837299	not calc	0.0806	0	0.0129	0.0023
	dev10air	573	20987345	not calc	0.0945	0.00116	0.01	0
	dev7air	574	20112038	not calc	0.0747	0	0.0287	0
	dev7air	575	19937399	not calc	0.0565	0.00581	0.0132	0
22/04/94	bl	577	19039075	0.0271	0	0	0.0124	0
	bl	578	49127768	0.0344	0	0.00517	0.0181	0
	dev7air	579	29340588	0.0872	0.00113	0.0304		
	bl	580	51295084	0.0197	0.00252	0.00458	0.0249	0
25/04/94	dev10m93	582	75247552	1.288	0	0.00461	0.0124	0
21/04/95	dev10m93	583	87320200	1.406	0	0.00453	0.0181	0.0027
	dev10m93	584	87652083	1.789	0.00184	0.00558	0.0249	0

	dev10m92	585	57992658	1.217	0	0.00137	0.0172	0
	dev10m92	586	49513620	1.283	0	0.0052	0	0
	dev10m92	587	26657088	1.94	0.00615	0.0061	0.0187	0.00223
	dev13m93	588	77163452	1.372	0.00592	0.00412	0.0312	0.00216
	dev13m93	589	68999975	1.317	0.00258	0.00357	0.024	0.00256
	dev13m93	590	70170469	1.7	0	0.00579	0.0218	0.00722
26/04/94	dev13m92	592	59616601	1.818	0	0.00586	0.0235	0.00554
	dev13m92	593	66218575	1.711	0.00415	0.00517	0.0286	0.00523
	dev13m92	594	66966329	1.319	0	0.00458	0.0157	0.00132
06/10/93	bl	602	55340394	not calc	0.0274	0.00162	0.00225	0.0334
	bl	603	52948754	not calc	0.0407	0	artefact	0.0309
	bl	604	49948476	not calc	0.0492	0	0	0.0444
	dev13a,air	605	61292822	not calc	0.0546	0	0	0.0342
	dev13a,air	606	60102005	not calc	0.0537	0.00335	0.00266	0.04
11/05/94	dev13a,air	608	38467947	not calc	0.0464	0.00778	0.00215	0.0425
	dev10a,air	609	53493047	not calc	0.0535	0	0	0.042
	dev10a,air	610	58882736	not calc	0.0239	0.0054	0.00222	0.0322
	dev10a,air	611	38367478	not calc	0.0545	0	0.00179	0.0498
	dev7a,air	612	46594784	not calc	0.0837	0	0.00254	0
	dev7a,air	613	58475898	not calc	0.0501	0	0.0001	0.0499
	dev7a,air	614	49778576	not calc	0.0762	0	0	0.0664
	dev13,1,air	615	66264555	not calc	0.0405	0.00102	0.00295	0.0274
	dev13,1,air	616	65473894	not calc	0.0395	0.00117	0	0.0384
	dev13,1,air	617	49568746	not calc	0.045	0.00134	0	0.0439
	dev13,2,air	618	40948957	not calc	0.0242	0.0019	0	0.0539
	dev13,2,air	619	57398309	not calc	0.0566	0.00345	0.00179	0.019
	dev13,2,air	620	47549048	not calc	0.0647	0.00226	0.0006	0.031
16/05/94	bl	622	84991567	not calc	0.00245	0	0	0
	bl	623	49921344	not calc	0.00564	0	0	0
	bl	624	52324199	not calc	0.011	0	0.00677	0.00985
	dev13,3,air	625	58340938	not calc	0.0634	0.00271	0.0009	0.0469
	dev13,3,air	626	46734834	not calc	0.0389	0.00709	0.0015	0.0237
	dev13,3,air	627	55847444	not calc	0.061	0.00311	0	0.0422
17/05/94	ban192	629	56439390	0.0383	0.00145	0	0	0.0204
09/05/95	ban192	630	57670518	0.0433	0.00353	0.00866	0	0
	ban192	631	60823993	0.0414	0	0.0101	artefact	0
	ban1,93	632	51421270	0.0378	0	0.00475	artefact	0.00333
	ban1,93	633	19956970	0.0353	0	0.00283	0	0
	ban1,93	634	39896880	0.0348	0.00065	0.00244	0	0.00113
	ban1a92	635	64219845	0.0285	0	0	0	0
	ban1a92	636	53510598	0.0158	0	0.00245	0	0
	ban1a92	637	47397999	0.032	0	0.00582	0.00116	0.0176
	ban1a,93	638	39510092	0.0384	0	0	0	0
	ban1a,93	639	16207825	0.0408	0	0	0	0
	ban1a,93	640	43953089	0.0348	0	0	0	0.0063
	ban1b,92	641	42398270	0.0616	0.00077	0.00654	0	0
18/05/94	ban1b,92	644	55788800	0.0596	0	0.00556	0	0
	ban1b,92	645	40358243	0.0618	0	0.00718	0	0
	ban1b,93	647	48739844	0.0526	0	0	0	0
	ban1b,93	648	39287303	0.0666	0.0015	0.0112	0	0
	ban1b,93	649	37593346	0.0829	0.0002	0	0	0
	ban1c,92	650	48212030	0.0669	0	0	0	0
	ban1c,92	651	32159907	0.063	0.00104	0.00371	0.00124	0
	ban1c,92	652	53669391	0.0983	0	0.00477	0.00113	0
	ban1c,93	653	59086694	0.0694	0	0.00632	0	0
	ban1c,93	654	39603937	0.116	0	0.00529	0.0016	artefact
	ban1c,93	655	62784449	0.13	0	0.00622	0	0

	ban2,92	656	36203454	0.0865	0	0.00444	0	0
	ban2,92	657	62661456	0.0982	0.0013	0.00694	0	0
	ban2,92	658	62564003	0.0981	0.0009	0.00379	0	0
19/05/94	ban2,93	664	65534446	0.169	0	0.008	0	0
	ban2,93	665	46366737	0.134	0	0.00342	0.00451	0.00022
	ban2,93	666	38295216	0.232	0.0026	0	0	0
	ban2a,92	667	59401272	0.194	0	0.00308	0	0.00076
	ban2a,92	668	12123374	0.158	0	0.00737	0	0
	ban2a,92	669	47635134	0.11	0	0	0	0
	ban2a,93	670	45409563	0.155	0	0.0043	0	0
	ban2a,93	671	45344864	0.139	0	0.00602	0	0
	ban2a,93	672	45247779	0.148	0.00588	0.00956	0	0
	ban2b,92	673	45466676	0.132	0.00534	0	0	0
	ban2b,92	674	14757182	0.13	0	0	0.0022	0
	ban2b,92	675	34414711	0.197	0	0.00543	0	0.00456
	ban2b,93	676	24170321	0.133	0	0.00858	0	0
	ban2b,93	677	40493853	0.121	0.00423	0.00524	0	0.00258
	ban2b,93	678	46632159	0.178	0	0.0128	0	0
20/05/94	ban2c,92	680	34536709	0.152	0.00881	0.0104	0.00729	0.00458
	ban2c,92	681	48209000	0.168	0.00485	0.0081	0	0.00788
	ban2c,92	682	36756080	0.156	0	0.00565	0	0.00323
	ban3,92	683	41875986	0.156	0.00284	0.00676	0	0
	ban3,92	684	75960925	0.163	0	0.00768	0	0.00194
	ban3,92	685	62259966	0.184	0	0.00835	0	0.0044
	ban2c,93	686	57470943	0.151	0	0.0101	0	0
	ban2c,93	687	69757513	0.23	0	0.00503	0	0
	ban2c,93	688	34428735	0.23	0	0.0103	0	0.00293
	ban3,93	689	47252673	0.151	0.0002	0.00281	0	0
	ban3,93	690	50042225	0.12	0	0.00896	0	0.00387
	ban3,93	691	51773199	0.215	0.0033	0.0132	0.00288	0.0046
	ban3a,92	692	55642778	0.184	0	0.00874	0	0
	ban3a,92	693	35256598	0.198	0	0.0115	0	0.00455
	ban3a,92	694	62320521	0.19	0	0.0113	0	0
	ban3a,93	695	57085397	0.224	0.0005	0	0	0.00573
	ban3a,93	696	37065993	0.194	0	artefact	0	0.00241
	ban3a,93	697	46098349	0.187	0	0.00971	0	0.00502
21/05/94	ban2b,93	699	56553316	0.41	0	0.00667	0	0
	ban2b,93	700	44313246	0.461	0	0.0109	0	0.00291
	ban2b,93	701	36314465	0.384	0	0.00567	0	0.00388
	ban3c,92	702	15262226	0.456	0.00359	0.0108	0	artefact
	ban3c,92	703	68277263	0.477	0	0.00903	0	0
	ban3c,92	704	46842040	0.489	0	0.0101	0	0.00576
	ban3c,93	705	51642833	0.458	0	0.00718	0	0
	ban3c,93	706	24452561	0.464	0	0.00933	0	0
	ban3c,93	707	59895010	0.458	0.0009	0.00853	artefact	0.00492
	ban92a	708	34937150	0.433	0.00167	0.00621	0	0.00237
	ban92a	709	50561149	0.411	0.00458	0.00907	0.0003	0
	ban92a	710	50369490	0.471	0	0.0069	0.0022	0.0186
	ban93a	711	55393659	0.47	0	0.00611	0	artefact
	ban93a	712	45972188	0.0487	0.0008	0	0	0.00518
	ban93a	714	47648628	0.057	0.0024	0.00566	0.00302	0
06/01/94	dev13,4,air	715	48374849	not calc	0.0634	0.00271	0	0.0277
	dev13,4,air	716	39908936	not calc	0.0389	0.00395	0.00165	0.0314
	dev13,5,air	717	38875465	not calc	0.0522	0.00127	0.0036	0.0459
	dev13,5,air	718	40093783	not calc	0.0709	0.00226	0.0017	0.0552
	dev13,6,air	719	41193844	not calc	0.052	0.00331	0.0005	0.0399
	dev13,6,air	720	42934847	not calc	0.0544	0.0024	0.00206	0.0276

	ban92a	721	49718655	0.0997	0	0.00771	0.000259	0
24/05/94	bl	723	62026005	1.007	0	0	0	0
23/05/94	bl	724	77729601	0.814	0	0	0	0
	bl	725	28050864	0.812	0	0.00278	0	0
	pon1w92	726	58808412	0.719	0	0	0.00099	0
	pon1w92	727	37115736	0.748	0	0	0	0
	pon1w93	728	26029969	0.783	0	0.00417	0	0
	pon1w93	729	45892890	0.767	0	0.00581	0	0
	pon1w93	730	37763531	0.851	0	0	0	0
	pon1w93	731	25466077	0.792	0	0.00612	0	0
	pon2w92	732	28056859	0.996	0.0004	0.00604	0	0
	pon2w92	733	67656587	0.905	0	0.00445	0.0003	0
	pon2w92	734	12764095	1.259	0	0.00835	artefact	0
25/05/94	pons2w93	736	25712735	0.896	0	0.00618	0.00035	0
	pons2w93	737	36494815	0.953	0	0	0.00014	0.00533
	pons2w93	739	26285233	0.932	0	0	0.000123	0
	pons3w92	740	41333673	1.123	0	0	0.00134	0
	pons3w92	741	52100062	1.006	0	0.00381	0	0
	pons3w92	742	21890254	0.783	0.00059	0	0.00222	0.0069
	pons3w93	743	13768882	0.885	0	0	0	0
	pons3w93	744	23133271	0.921	0	0.00293	0.0005	0
	pons3w93	745	60912812	0.887	0	0.00391	0.00032	0
	pons4w92	746	45662640	0.915	0	0.00466	0	0
	pons4w92	747	47025995	0.904	0	0	0.0002	0.00208
	pons4w92	748	46597091	0.937	0	0	0	0
	pons4w93	749	25318257	1.19	0	0.00696	0	0
	pons4w93	750	36677360	1.091	0	0.00221	0	0
	pons4w93	751	28540351	0.904	0	0.00234	0	0
26/05/94	pons1c92	753	17505990	0.952	0.00029	0.00583	0.0011	0
	pons1c92	754	26229697	1.03	0.00106	0.00437	0	0
	pons1c92	755	25463865	0.941	0	0.00475	0	0
	pons1c93	756	25431617	1.026	0.00694	0.00676	0	0
	pons1c93	757	25579323	0.99	0	0	0	0.00696
	pons1c93	758	25033713	0.939	0	0	0	0
	pons2c92	759	36701107	1.213	0.00226	0.00598	0.0008	0
	pons2c92	760	26241405	1.211	0.00223	0.0055	0.001	0
	pons2c92	761	34569784	1.082	0	0.00253	0.00212	0
	pons2c93	762	10069556	1.239	0	0.00216	0	0
	pons2c93	763	13011107	1.146	0	artefact	0	0
	pons2c93	764	15676670	0.955	0.0084	0.00335	0.00201	0
	pons3c92	765	28697227	1.153	0	0.0091	0	0
	pons3c92	766	49978651	1.017	0	0.00457	0	0
27/05/94	pons3c92	768	26037587	1.053	0	0.00412	0	0
	pons3c93	769	25652742	1.04	0	0.00389	0	0
	pons3c93	770	18637270	1.133	0	0.000524	0.00104	0
	pons3c93	771	35348873	1.249	0.00172	0.00427	0.0007	0.00492
	pons1e92	772	24292049	1.26	0	0	0.000622	0
	pons1e92	773	13383120	1.054	0	0.00375	0	0
	pons1e92	774	42392164	1.059	0	0	0	0
	pons1e93	775	28735012	1.05	0	0.00346	0.00272	0
	pons1e93	776	50208529	1.025	0	0.00494	0	0
	pons1e93	777	25451319	1.048	0	0.00283	0	0
	pons2e92	778	25622629	1.081	0	0	0	0
	pons2e92	779	46091513	1.083	0	0.00791	0	0
	pons2e92	780	16833363	1.17	0	0.00475	0	0
	ponsw92a	781	17365778	1.149	0	0.000424	0	0
	ponsw92a	782	15501406	1.296	0.000921	0.00198	0.00114	0

	ponsw92a	783	19525425	1.214	0	0.00646	0	0
28/05/94	ponsw93a	785	19691050	1.072	0	0.00533	0	0
	ponsw93a	787	24199125	1.127	0	0.00419	0.0001	0
	ponsw93a	788	15379351	1.356	0	0	0.00077	0
	ponsc92a	789	36893341	1.169	0	0.00195	0.0007	0
	ponsc92a	790	14863082	1.211	0	0.00587	0.00243	0.00145
	ponsc92a	791	27086060	1.139	0.00114	0.00384	0	0
	ponsc93a	792	56728462	1.079	0	0.00307	0.000202	0
	ponsc93a	793	25056315	1.262	0	0.00582	0.00169	0
	ponsc93a	794	46599322	1.133	0.0039	0.00415	0	0
	pons2e93	795	45673951	1.337	0	0.00715	0	0
	pons2e93	796	66259867	1.182	0	0.0048	0	0
	pons2e93	797	45462140	1.132	0	0.00368	0	0.00246
	pons3e92	798	69874289	1.175	0	0	0	0
	pons3e92	799	11231451	0.992	0	0.00486	0	0
	pons3e92	801	15266113	1.084	0	0	0	0.00388
	pons3e93	802	27382540	1.274	0	0.00172	0.00643	0
	pons3e93	803	46783893	1.211	0	0.00472	0	0
	pons3e93	804	26322982	1.077	0	0.00276	0	0
30/05/94	pons4e92	806	45837064	1.066	0	0.00535	0	0.0029
	pons4e92	807	38058194	1.094	0	0.00316	0.00257	0.0005
	pons4e92	808	26688210	1.254	0	0	0	0
	pons4e93	809	27520506	1.272	0	0.00486	0	0
	pons4e93	810	55126127	1.264	0	0	0	0
	pons4e93	811	25158960	1.077	0	artefact	0	0
	ponse92a	812	35173595	1.139	0	0.00507	0	0
	ponse92a	813	46913724	1.613	0	0.00384	0	0
	ponse92a	814	19482649	1.558	0	0.00345	0	0
	ponse93a	815	29723256	1.125	0	0.00353	0	0
	ponse93a	816	11601582	1.138	0	0.00509	0.0004	0.00346
	ponse93a	817	25307957	1.298	0	0	0.00138	0
06/06/94	bl	819	26113182	3.73	0	0.00363	0	0
31/05/95	bl	820	34090029	5.516	0.000468	0.00341	0.00034	0.00145
	bl	821	13733041	3.247	0	0.00367	0	0
	dev110m92	822	16501304	3.581	0	0.00269	0	0
	dev110m92	823	18919934	3.689	0.000285	0.00542	0	0
	dev110m92	824	38655415	3.269	0.000274	0.00102	0	artefact
	dev110m93	825	28169351	3.548	0	0	0	0
	dev110m93	826	67891057	3.3	0	0	0	0
	dev110m93	827	45770626	3.572	0.00126	0.00353	0	0
	dev210m92	828	51410223	6.482	0	0.00152	0	0
	dev210m92	829	59284878	3.244	0	0.00483	0	0.0015
	dev210m92	830	17581026	3.327	0	0.00405	0.00004	0
	dev210m93	831	34902707	4.89	0	0.00345	0.00046	0.00066
	dev210m93	832	49716584	3.785	0	0.00398	0.0001	0
	dev210m93	833	10825771	4.044	0	0.00544	0	0
07/06/94	dev310m92	835	26610052	3.884	0	0.00277	0	0
	dev310m92	836	27972631	4.216	0.00194	0.00544	0	0
	dev310m92	837	35529820	3.37	0.0023	0.00685	0.00002	0
	dev310m93	838	48450228	3.959	0	0.0069	0	0
	dev310m93	839	37013916	2.999	0	0.00406	0	0.00291
	dev310m93	840	25543453	3.089	0.00014	0.00317	0.000105	0
	dev410m92	841	17476319	4.759	0	0.00698	0.00011	0
	dev410m92	842	47093582	4.101	0.00225	0.003	0.0001	0
	dev410m92	843	62547398	4.321	0	0.00373	0	0
	dev410m92	844	60453711	3.815	0	0.00547	0	0
	dev410m92	845	49483300	4.782	0	0.00522	0	0

	dev410m92	846	45280872	3.519	0.000768	0.00367	0	0
	dev10m92a	847	45962602	4.46	0.00225	0.00571	0	0.00427
	dev10m92a	848	35237825	4.196	0	0.00509	0	0
	dev10m92a	849	36296533	4.29	0	0.00402	0.00004	0
08/06/94	dev10m93a	851	34519870	5.389	0	0.00569	0.00005	0.00151
	dev10m93a	852	58518460	4.518	0	0.00266	0.00003	0
	dev10m93a	853	38613496	3.541	0.000943	0.00337	0	0.00415
	dev510m92	854	50896113	4.344	0.0021	0.00237	0	0
	dev510m92	855	57244024	5.02	0	0.00412	0	artefact
	dev510m92	856	63011273	4.018	0	0.00395	0	0.00405
	dev510m93	857	52527630	4.255	0	0.00447	0	0
	dev510m93	858	25792936	4.595	0	0.00361	0	0.00436
	dev510m93	859	34103470	4.758	0.00058	0.00494	0	0
	dev610m92	860	27322468	4.519	0	0.00576	0	0
	dev610m92	861	68636765	4.618	0	0.00521	0	0
	dev610m92	862	53332832	5.132	0	0.00514	0	0.00608
	dev610m93	863	43651977	4.92	0	0.00347	0.00009	0
	dev610m93	864	46137809	5.505	0	0.00549	0.000106	0
	dev610m93	865	43117070	5.014	0	0.00379	0	0
09/06/94	dev710m93	867	48609265	16.34	0.00106	0.00464	0.0001	0.09033
	dev710m93	868	25702636	4.925	0	0.00506	0	0.0039
	dev710m93	869	25681126	5.421	0	0.005	0	0.00292
	dev710m92	870	34999015	5.021	0	0.00761	0	artefact
	dev710m92	871	46971208	5.541	0	0.00706	0	0.00804
	dev710m92	872	34655761	5.057	0	0.00732	0	0
	dev810m92	873	60613932	4.85	0	0.00989	0.00008	0
	dev810m92	874	54616283	4.643	0	0	0	0
	dev810m92	875	36526589	4.504	0	0.00597	artefact	0
10/06/94	dev810m93	877	29022489	6.75	0	0.00295	0	0
	dev810m93	878	42308314	5.596	0	0.00645	0	0.00311
	dev810m93	879	22613497	5.32	0.000333	0.00634	0	0
	dev910m92	880	25756668	5.277	0.000123	0.00664	0	0
	dev910m92	881	43201474	6.072	0	0.00936	0	0.0043
	dev910m92	882	36670969	4.587	0	0.00568	0	0
	dev213m92	883	34135276	8.437	0	0.00951	0	0
	dev213m92	884	45600247	6.866	0	0.00603	0.00001	0
	dev213m92	885	35819656	6.814	0	0.00714	0.0001	0.00262
13/06/94	dev13m92a	887	56041439	8.76	0	0.0107	0	0
	dev13m92a	888	72816400	8.465	0	0.0113	0.00009	0
	dev13m92a	889	11608543	7.462	0	0.00864	0	0.00273
	dev113m93	890	36485955	8.96	0	0.0115	0.00009	0
	dev113m93	891	35805795	7.567	0.000669	0.0138	0.000022	artefact
	dev113m93	892	50046977	12.37	0.00178	0.0166	0	0.00404
14/06/94	dev13m93a	899	13459962	8.501	0.000282	0.00664	0.0001	0
	dev13m93a	900	47235397	13.16	0.00423	0.00782	0	0.00159
	dev13m93a	901	45495755	1.774	0.00385	0.00392	0.000099	0.00474
	dev313m92	902	37676164	0.00627	0	0.00389	0	0
	dev313m92	903	35541542	0.00889	0	0	0.000013	0
	dev313m92	904	22701170	0.0143	0	0	0.00006	0.00265
22/06/94	dev213m93	906	37686777	0.013	0	0.00375	0	0
	dev213m93	907	45729987	0.305	0	0.00551	0	0.0037
	dev213m93	908	57574448	0.038	0.003	0	0	0.00336
	dev313m93	909	57176185	0.0193	0.000407	0	0	0.00408
	dev313m93	910	26568994	0.0222	0.00411	0.00498	0	0
	dev910m93	911	45767923	0.165	0	0	0	0
	dev910m93	912	24384346	0.165	0	0.00302	0	0
	dev910m93	913	46619991	0.0941	0.00004	0.00493	0	0

23/06/94	dev1010,92	915	59471311	0.0995	0	0	0	0
	dev1010,92	916	35644367	0.0916	0	0.000641	0	0
	dev1010,92	917	60460007	0.106	0	0.00281	0	artefact
	dev1010,93	918	35347768	0.125	0	0	artefact	0.00619
	dev1010,93	922	61625276	0.099	0	0.00382	0	0
	dev1010,93	923	63105478	0.0933	0	0	0.0002	0
	dev1110,92	924	14609790	0.0823	0.00047	0	0	0.0045
	dev1110,92	925	35769785	0.06	0.00108	0.00104	artefact	0
	dev1110,92	926	65040501	0.0703	0	0.00477	0.0001	0.00404
24/06/94	dev1110,93	928	32189555	0.112	0	0	0	0
	dev1110,93	929	24879234	0.205	0.00066	0.00531	0.00011	artefact
	dev1110,93	930	46096890	0.125	0.00084	0.00494	0.000118	0.00399
	dev13m93a	931	37027916	2.958	0.000505	0.0158	0	0
	dev1210,92	933	47750577	0.159	0	0.00455	0	0
	dev1210,92	934	12137451	0.171	0	0.00575	0	0
	dev1210,92	935	13018266	0.184	0.0016	0.00186	0	0
	dev1210,93	936	22180068	0.156	0	0	0	0
	dev1210,93	937	38413549	0.141	0	0	0	0
	dev1210,93	938	28795620	0.134	0	0	0	0
27/06/94	bl	940	28394754	not calc	0	0	0.00247	0
10/02/94	bl	941	30193845	not calc	0	0	artefact	0.0078
	bl	942	37834944	not calc	0.0034	0	0	0
	dev13,air	943	45372893	not calc	0.0448	0	0.002	0
	dev13,air	944	44483947	not calc	0.0143	0	0.00984	0.0138
	dev13,air	945	30947834	not calc	0.0778	0.0057	0.0156	0
	dev10,air	946	46383094	not calc	0	0.00109	0	0
	dev10,air	947	36383954	not calc	0	0.0025	0.0097	0
	dev10,air	948	43937846	not calc	0	0	0.0066	0
	dev7,air	949	29099856	not calc	0.00592	0	0	0
	dev7,air	950	38276436	not calc	0	0.0012	0.00157	0
	dev7,air	951	47839837	not calc	0	0.0008	0.00466	0
30/06/94	bl	953	42348456	not calc	0.00451	0.00323	0.00641	0
16/02/94	bl	954	38947565	not calc	0	0.00405	0	0
	bl	955	34947464	not calc	0.00395	0.00426	0	0
	ponswair	956	26734954	not calc	0.0388	0.00401	0.00321	0.00429
	ponswair	957	29374654	not calc	0.0313	0.0058	0.0159	0
	ponswair	958	49384757	not calc	0	0.00676	0.0098	0
	ponscair	959	33382937	not calc	0.0193	0.0129	0.00365	0.00192
	ponscair	960	34398478	not calc	0.0663	0.00708	0.0273	0.00998
	ponscair	961	33938933	not calc	0.0286	0.0205	0.0227	0
	ponseair	962	43228273	not calc	0.0398	0.00402	artefact	0.00192
	ponseair	963	42829304	not calc	0.0343	0.0115	0.0206	0.00569
	ponseair	964	30938373	not calc	0	0.00782	0.0138	0
11/07/94	bl	966	48237363	not calc	0	0	0	0
03/03/94	bl	967	30283744	not calc	0.0013	0	0	0
	bl	968	47372823	not calc	0.005	0	0	0.0132
	ban1air	969	20034836	not calc	0	0.00269	0	0
	ban1air	970	23937363	not calc	0.0045	0	0.00109	0.0114
	ban1air	971	30292383	not calc	0	0.00742	0.00024	0
	ban2air	972	46327838	not calc	0	0	0.002	0
	ban2air	973	43290398	not calc	0.0087	0	0.00055	0.019
	ban2air	974	34636367	not calc	0	0.00546	0	0
	ban3air	975	55034894	not calc	0	0	0	0
	ban3air	976	29485743	not calc	0.00221	0.00518	0	0.00307
	ban3air	977	52934784	not calc	0	0	0.00382	0
12/07/94	bl	979	49304985	not calc	0	0.00222	0	0
31/05/94	bl	980	48889833	not calc	0	0.00216	0.00357	0

	bl	981	20485474	not calc	0	0.0018	0.0009	0
	dev13air	982	30494874	not calc	0	artefact	0	0
	dev13air	983	40594744	not calc	0	0.00358	0	0
	dev13air	984	41849373	not calc	0.0167	0.00357	0	0.00141
	dev10air	985	48393837	not calc	0.0014	0.0006	0.00248	0
	dev10air	986	47777738	not calc	0	0.00318	0	0
	dev10air	987	46859434	not calc	0	0.004	0.00357	0
	dev7air	988	46239383	not calc	0	0.00185	0	0
	dev7air	989	44221091	not calc	0	0.00188	0	0
	dev7air	990	42839437	not calc	0	0.00303	0	0
13/07/94	bl	992	48393033	not calc	0	0.00783	0.00783	0
23/06/93	bl	993	35323404	not calc	0	0	0	0
	bl	994	53049854	not calc	0.00242	0.0129	0.0129	0.007
	ponswair	995	36374855	not calc	0	0	0.0118	0.00401
	ponswair	996	44943644	not calc	0	0.00301	0.00757	0
	ponswair	997	31523322	not calc	0.00107	0.00246	0.014	0
	ponscair	998	32783462	not calc	0	0.00279	0.00887	0
	poncair	999	43646744	not calc	0.00303	0.00139	0.013	0
	ponscair	1000	35262782	not calc	0	0.00364	0.00961	0.0046
	ponseair	1001	64738333	not calc	0	0.00416	0.0187	0
	ponseair	1002	62992019	not calc	0	0.00304	0.00991	0
	ponseair	1003	33737838	not calc	0	0.00239	0.00494	0
14/07/94	bl	1006	47383944	not calc	0.038	0.00368	0.00622	0
09/06/94	bl	1007	62352633	not calc	0.0043	0.00599	0.00124	0
	bl	1008	60001234	not calc	0	0.00385	0.00767	0.00322
	ban1air	1009	34747489	not calc	0.0312	0.00851	0.00476	0.0109
	ban1air	1010	53738444	not calc	artefact	0.00364	0.0097	0
	ban1air	1011	55556474	not calc	0	0	0.0125	0
	ban2air	1012	45438387	not calc	0	0.0031	0.0041	0
	ban2air	1013	48948444	not calc	artefact	0.00188	0.0137	0.00768
	ban3air	1014	51937834	not calc	0.0045	0.0067	0.00638	0
	ban3air	1015	50283000	not calc	0.0084	0.00292	0.00752	0
	ban3air	1016	37448333	not calc	0	0	0.0067	0
18/07/94	bl	1019	70018273	not calc	0	0.00437	0.00714	0
27/06/94	bl	1020	69363738	not calc	0.00206	0	0.0122	0
	bl	1021	67738333	not calc	0.00068	0.00752	0.0106	0.00088
	dev13air	1022	62627838	not calc	0	0.000275	0.00782	0
	dev13air	1023	43253644	not calc	0	0.00394	0.015	0.00175
	dev13air	1024	32833643	not calc	0	artefact	0.0124	0
	dev10air	1025	36238348	not calc	0	0.00126	0.0101	0
	dev10air	1026	57575555	not calc	0.00125	0	0.0149	0.00209
	dev10air	1027	66767444	not calc	0	0	0.0163	0
	dev7air	1028	46999685	not calc	0	0.00495	0.0125	0
	dev7air	1029	57699966	not calc	0.00133	0.00514	0.0139	0
	dev7air	1030	65646922	not calc	0	0.00661	0.013	0
Calibration data								
Calibration data over all six and three stage calibrations. 180pg check values are also included.								
Date	**pg/inj**	**Run no.**	**Internal standard peak area**	**CHCl3 ISR**	**C2H3Cl3 ISR**	**CCl4 ISR**	**C2HCl3 ISR**	**C2Cl4 ISR**
07/06/93	180	89	54983275	0.128	0.083	0.31	0.025	0.12
08/06/93	180	95	48300923	0.13	0.071	0.325	0.022	0.125
09/06/93	180	100	76391744	0.112	0.078	0.319	0.034	0.123

10/06/93	180	103	23548790	0.145	0.082	0.331	0.03	0.13
21/06/93	180	114	32654872	0.119	0.066	0.32	0.028	0.135
22/06/93	180	120	32764511	0.114	0.077	0.323	0.029	0.136
24/06/93	180	130	62189534	0.109	0.063	0.307	0.035	0.121
25/06/93	500	140	22361108	0.267	0.211	1.006	0.105	0.294
25/06/93	500	141	43187435	0.263	0.232	1.002	0.096	0.306
25/06/93	500	142	19456220	0.258	0.22	0.996	0.11	0.284
25/06/93	360	143	18398888	0.189	0.15	0.7	0.075	0.24
25/06/93	360	144	55521032	0.196	0.147	0.693	0.079	0.225
25/06/93	360	145	32443356	0.18	0.156	0.71	0.085	0.233
25/06/93	270	146	38725689	0.16	0.112	0.503	0.056	0.186
25/06/93	270	147	60112843	0.157	0.106	0.515	0.052	0.174
25/06/93	270	148	11265476	0.155	0.115	0.497	0.066	0.18
25/06/93	180	149	13479984	0.113	0.073	0.306	0.037	0.128
25/06/93	180	150	24354436	0.105	0.068	0.322	0.029	0.139
25/06/93	180	151	56873321	0.124	0.082	0.312	0.03	0.122
25/06/93	90	152	47953346	0.065	0.022	0.126	0.019	0.103
25/06/93	90	153	22154489	0.071	0.018	0.114	0.015	0.088
25/06/93	90	154	24654432	0.08	0.029	0.13	0.011	0.096
25/06/93	40	155	17769334	0.042	0.01	0.07	0.004	0.03
25/06/93	40	156	13984432	0.036	0.008	0.066	0.005	0.029
25/06/93	40	157	29873011	0.03	0.013	0.059	0.001	0.02
29/06/93	180	169	35419983	0.117	0.083	0.316	0.032	0.13
30/06/93	180	180	41109332	0.12	0.072	0.322	0.027	0.125
28/09/93	180	215	11564487	0.122	0.069	0.3	0.037	0.137
28/09/93	180	216	12874439	0.098	0.079	0.315	0.029	0.128
28/09/93	90	217	23987662	0.066	0.019	0.118	0.019	0.105
28/09/93	90	218	14655587	0.067	0.021	0.089	0.011	0.09
28/09/93	40	219	65729871	0.042	0.013	0.069	0.002	0.035
28/09/93	40	220	10967329	0.036	0.009	0.061	0.006	0.027
17/11/93	180	221	27659823	0.127	0.075	0.315	0.031	0.136
18/11/93	500	222	31096338	0.271	0.21	1.007	0.116	0.292
18/11/93	500	223	54236510	0.255	0.209	1.03	0.103	0.288
18/11/93	500	224	30002753	0.26	0.222	1.1	0.099	0.312
18/11/93	360	225	48711298	0.194	0.155	0.714	0.087	0.222
18/11/93	360	226	23871130	0.179	0.169	0.69	0.089	0.231
18/11/93	360	227	28964359	0.184	0.142	0.705	0.07	0.24
18/11/93	270	228	31214335	0.159	0.119	0.521	0.055	0.164
18/11/93	270	229	43654562	0.167	0.11	0.504	0.063	0.178
18/11/93	270	230	22235654	0.147	0.127	0.516	0.059	0.181
18/11/93	180	232	14776777	0.11	0.077	0.33	0.038	0.116
18/11/93	180	233	12754467	0.092	0.071	0.3	0.034	0.132
18/11/93	180	234	23546478	0.07	0.06	0.313	0.04	0.125
18/11/93	90	235	68110023	0.067	0.027	0.117	0.009	0.082
18/11/93	90	236	19823843	0.076	0.021	0.134	0.014	0.1
18/11/93	90	237	32597866	0.064	0.03	0.12	0.02	0.094
18/11/93	40	238	69388827	0.028	0.013	0.069	0.001	0.021
18/11/93	40	239	36288741	0.032	0.019	0.075	0.004	0.028
18/11/93	40	240	11836642	0.035	0.007	0.077	0.005	0.035
22/11/93	180	244	10753325	0.087	0.066	0.302	0.029	0.118
24/11/93	180	248	56983455	0.109	0.07	0.317	0.033	0.13
25/11/93	180	256	31100325	0.099	0.08	0.301	0.028	0.127
30/11/93	180	263	12274901	0.108	0.082	0.3	0.025	0.128
01/12/93	180	269	16458732	0.096	0.085	0.304	0.029	0.137
02/12/93	180	279	16678444	0.122	0.064	0.324	0.024	0.138
03/12/93	180	286	12853687	0.102	0.073	0.297	0.032	0.126
07/12/93	180	312	12439990	0.134	0.072	0.301	0.039	0.124

13/12/93	180	313	13576328	0.127	0.064	0.317	0.027	0.133
13/12/93	180	314	47773628	0.109	0.08	0.305	0.032	0.139
13/12/93	90	315	22281001	0.087	0.035	0.126	0.009	0.082
13/12/93	90	316	28841034	0.066	0.021	0.114	0.017	0.099
13/12/93	40	317	50185421	0.033	0.012	0.061	0.005	0.0231
13/12/93	40	318	28762213	0.05	0.01	0.055	0.002	0.042
05/01/94	180	319	30012443	0.105	0.081	0.304	0.028	0.14
06/01/94	180	320	40783168	0.104	0.084	0.32	0.03	0.136
07/01/94	180	321	23421165	0.087	0.078	0.316	0.035	0.132
10/01/94	180	322	12687566	0.098	0.08	0.327	0.027	0.135
11/01/94	180	323	10992873	0.118	0.069	0.316	0.04	0.129
12/01/94	180	324	12654100	0.112	0.073	0.323	0.032	0.119
13/01/94	180	325	70021464	0.125	0.075	0.318	0.036	0.125
14/01/94	180	327	16793250	0.101	0.084	0.309	0.03	0.127
17/01/94	180	335	35447921	0.108	0.076	0.312	0.027	0.135
18/01/94	500	377	47210992	0.26	0.23	0.995	0.098	0.284
18/01/94	500	378	18326877	0.253	0.225	0.9	0.114	0.311
18/01/94	500	379	17620922	0.259	0.217	1.04	0.108	0.327
18/01/94	360	380	54311882	0.18	0.14	0.721	0.074	0.23
18/01/94	360	381	43652100	0.192	0.145	0.706	0.076	0.217
18/01/94	360	382	10942337	0.175	0.157	0.698	0.088	0.245
18/01/94	270	383	28354022	0.146	0.103	0.502	0.052	0.168
18/01/94	270	384	20730421	0.16	0.116	0.515	0.047	0.171
18/01/94	270	385	48329003	0.157	0.109	0.49	0.045	0.185
18/01/94	180	386	57843500	0.111	0.079	0.327	0.04	0.147
18/01/94	180	387	11764912	0.08	0.071	0.316	0.029	0.109
18/01/94	180	388	37923404	0.085	0.089	0.323	0.031	0.125
18/01/94	90	389	54723792	0.056	0.029	0.113	0.016	0.096
18/01/94	90	390	44446001	0.073	0.025	0.13	0.011	0.085
18/01/94	90	391	30926708	0.066	0.017	0.124	0.017	0.112
18/01/94	40	392	23276874	0.027	0.009	0.075	0.008	0.032
18/01/94	40	393	30760702	0.03	0.01	0.066	0.005	0.024
18/01/94	40	394	11209087	0.041	0.006	0.083	0.01	0.019
19/01/94	180	395	30457376	0.096	0.059	0.32	0.029	0.143
20/01/94	180	396	45243447	0.089	0.063	0.321	0.034	0.142
21/01/94	180	397	56437865	0.106	0.072	0.304	0.037	0.134
25/01/94	180	398	46201020	0.109	0.075	0.322	0.033	0.129
26/01/94	180	400	22368711	0.111	0.08	0.324	0.026	0.119
27/01/94	180	406	47939917	0.12	0.081	0.316	0.022	0.126
28/01/94	180	411	38771773	0.107	0.077	0.314	0.026	0.135
06/02/94	180	412	40921886	0.109	0.07	0.327	0.033	0.125
08/02/94	180	413	40826474	0.122	0.064	0.321	0.039	0.123
11/02/94	180	414	22472921	0.108	0.066	0.316	0.026	0.128
14/02/94	180	418	56398275	0.099	0.08	0.308	0.034	0.137
15/02/94	180	428	54728466	0.107	0.081	0.31	0.037	0.13
17/02/94	180	432	46657485	0.102	0.083	0.319	0.041	0.14
18/02/94	180	435	49382058	0.129	0.063	0.306	0.042	0.125
18/02/94	180	436	57403850	0.1	0.082	0.329	0.036	0.132
18/02/94	90	437	36498205	0.079	0.019	0.13	0.016	0.094
18/02/94	90	438	67493840	0.063	0.037	0.09	0.014	0.09
18/02/94	40	439	28597940	0.041	0.013	0.074	0.005	0.042
18/02/94	40	440	17395068	0.031	0.005	0.079	0.008	0.03
21/02/94	180	450	26937585	0.129	0.072	0.328	0.04	0.133
22/02/94	180	457	38477440	0.122	0.076	0.325	0.038	0.126
23/02/94	180	465	37282845	0.108	0.071	0.33	0.03	0.13
24/02/94	180	470	22263534	0.11	0.065	0.321	0.025	0.123
02/03/94	180	479	29478849	0.097	0.079	0.307	0.023	0.126

03/03/94	180	490	23638491	0.096	0.08	0.315	0.036	0.139
04/03/94	180	495	30930398	0.11	0.084	0.311	0.028	0.121
07/03/94	180	504	27279103	0.121	0.085	0.324	0.025	0.126
08/03/94	180	509	28908478	0.108	0.067	0.32	0.029	0.129
09/03/94	180	519	29238470	0.103	0.065	0.326	0.03	0.125
10/03/94	180	524	22043958	0.097	0.071	0.308	0.025	0.13
11/03/94	180	525	64839465	0.099	0.079	0.301	0.039	0.134
14/03/94	180	526	14365374	0.132	0.086	0.32	0.033	0.115
14/03/94	180	527	33747483	0.127	0.069	0.306	0.027	0.137
14/03/94	90	528	22368455	0.1	0.032	0.121	0.013	0.075
14/03/94	90	529	62837484	0.085	0.017	0.12	0.02	0.083
14/03/94	40	530	36287573	0.047	0.012	0.071	0.003	0.038
14/03/94	40	531	43893934	0.05	0.001	0.054	0.001	0.026
15/04/94	180	532	19484833	0.098	0.059	0.33	0.037	0.13
18/04/94	500	536	20475849	0.259	0.212	0.993	0.113	0.32
18/04/94	500	537	40247484	0.262	0.23	1.09	0.11	0.296
18/04/94	500	538	54749294	0.27	0.245	0.982	0.092	0.314
18/04/94	360	539	56473934	0.196	0.159	0.697	0.069	0.205
18/04/94	360	540	39575849	0.182	0.168	0.685	0.074	0.228
18/04/94	360	541	59475894	0.179	0.137	0.713	0.08	0.21
18/04/94	270	542	13543744	0.143	0.13	0.516	0.06	0.167
18/04/94	270	543	19375219	0.15	0.102	0.497	0.047	0.161
18/04/94	270	544	79582922	0.162	0.118	0.49	0.051	0.155
18/04/94	180	545	45748486	0.085	0.075	0.3	0.03	0.142
18/04/94	180	546	33848484	0.096	0.06	0.315	0.041	0.112
18/04/94	180	547	22253638	0.091	0.053	0.331	0.032	0.133
18/04/94	90	548	19448999	0.071	0.022	0.134	0.02	0.094
18/04/94	90	549	22938328	0.062	0.031	0.119	0.013	0.1
18/04/94	90	560	56575580	0.065	0.025	0.126	0.018	0.082
18/04/94	40	561	20938002	0.029	0.008	0.078	0.008	0.027
18/04/94	40	562	16389038	0.035	0.017	0.073	0.001	0.036
18/04/94	40	563	18474663	0.024	0.001	0.082	0.004	0.023
19/04/94	180	564	19376662	0.087	0.062	0.329	0.032	0.14
20/04/94	180	565	57393843	0.088	0.073	0.321	0.043	0.129
22/04/94	180	576	67484946	0.103	0.082	0.325	0.039	0.116
25/04/94	180	581	27400344	0.105	0.066	0.325	0.035	0.122
26/04/94	180	591	48201901	0.107	0.073	0.32	0.026	0.132
27/04/94	180	595	37373018	0.112	0.08	0.327	0.032	0.133
28/04/94	180	597	63883829	0.114	0.084	0.321	0.027	0.118
11/05/94	180	601	50004882	0.116	0.064	0.326	0.031	0.135
12/05/94	180	607	17356384	0.104	0.072	0.316	0.036	0.122
16/05/94	180	621	19374892	0.105	0.079	0.324	0.033	0.123
17/05/94	180	628	39274955	0.097	0.079	0.309	0.03	0.116
18/05/94	180	643	55059438	0.106	0.081	0.31	0.027	0.138
19/05/94	180	659	30038062	0.099	0.083	0.328	0.032	0.128
19/05/94	180	660	34527464	0.096	0.078	0.315	0.038	0.119
19/05/94	90	661	54558473	0.071	0.03	0.09	0.014	0.079
19/05/94	90	662	48355784	0.062	0.012	0.088	0.02	0.096
19/05/94	40	663	29873623	0.029	0.017	0.065	0.002	0.029
19/05/94	40	664	29837289	0.024	0.01	0.069	0.004	0.033
20/05/94	180	679	68982986	0.103	0.068	0.325	0.035	0.135
21/05/94	180	698	55869885	0.096	0.07	0.329	0.022	0.131
24/05/94	180	722	29202857	0.099	0.079	0.319	0.024	0.121
25/05/94	180	735	70059505	0.114	0.088	0.312	0.031	0.12
26/05/94	180	752	12928900	0.115	0.083	0.304	0.026	0.1137
27/05/94	180	767	57489303	0.117	0.075	0.303	0.034	0.129
28/05/94	180	784	49303038	0.11	0.07	0.317	0.028	0.115

30/05/94	180	805	29494038	0.102	0.072	0.314	0.021	0.124
06/06/94	180	818	50504848	0.096	0.069	0.322	0.037	0.116
07/06/94	180	834	20229227	0.095	0.054	0.301	0.033	0.124
08/06/94	180	850	19292792	0.118	0.072	0.336	0.026	0.132
09/06/94	180	866	39303833	0.114	0.063	0.332	0.025	0.118
10/06/94	180	876	69666829	0.114	0.081	0.306	0.04	0.123
13/06/94	180	886	72198343	0.101	0.079	0.314	0.028	0.136
14/06/94	180	893	37293993	0.117	0.052	0.34	0.03	0.124
14/06/94	180	894	62000287	0.101	0.07	0.323	0.033	0.122
14/06/94	90	895	52867444	0.073	0.029	0.093	0.015	0.081
14/06/94	90	896	34598645	0.068	0.018	0.116	0.019	0.089
14/06/94	40	897	54553565	0.038	0.015	0.08	0.003	0.031
14/06/94	40	898	26117253	0.021	0.003	0.073	0.002	0.024
22/06/94	180	905	41947634	0.087	0.065	0.32	0.024	0.132
23/06/94	180	914	67361136	0.089	0.069	0.322	0.023	0.14
24/06/94	180	927	40291749	0.099	0.059	0.316	0.036	0.139
27/06/94	180	939	41940846	0.108	0.07	0.319	0.037	0.137
30/06/94	180	952	67849130	0.113	0.073	0.325	0.039	0.133
11/07/94	180	965	63819047	0.115	0.083	0.327	0.044	0.126
12/07/94	180	978	25485982	0.126	0.089	0.323	0.034	0.12
13/07/94	180	991	29573922	0.128	0.074	0.308	0.03	0.14
14/07/94	180	1005	50174528	0.125	0.065	0.33	0.029	0.127
15/07/94	180	1017	58146591	0.122	0.072	0.314	0.025	0.137
18/07/94	180	1018	30174631	0.11	0.08	0.325	0.023	0.136
19/07/94	180	1031	36490284	0.121	0.066	0.309	0.023	0.131
20/07/94	500	1044	46289039	0.249	0.206	0.9	0.102	0.3
20/07/94	500	1045	36777383	0.23	0.223	0.992	0.117	0.295
20/07/94	500	1046	21974033	0.261	0.215	1.002	0.096	0.281
20/07/94	360	1047	57103036	0.177	0.153	0.714	0.089	0.219
20/07/94	360	1048	27003647	0.184	0.145	0.72	0.077	0.206
20/07/94	360	1049	55293365	0.195	0.134	0.701	0.074	0.239
20/07/94	270	1050	27306303	0.153	0.129	0.495	0.056	0.165
20/07/94	270	1051	24362304	0.164	0.111	0.523	0.049	0.183
20/07/94	270	1052	56403533	0.149	0.114	0.512	0.061	0.188
20/07/94	180	1053	71027352	0.099	0.078	0.321	0.028	0.129
20/07/94	180	1054	23130463	0.115	0.064	0.306	0.022	0.127
20/07/94	180	1055	12222439	0.112	0.086	0.316	0.029	0.122
20/07/94	90	1056	46384839	0.067	0.014	0.103	0.018	0.097
20/07/94	90	1057	28475593	0.055	0.027	0.112	0.021	0.091
20/07/94	90	1058	30027465	0.053	0.035	0.124	0.013	0.083
20/07/94	40	1059	37463920	0.029	0.009	0.074	0.004	0.043
20/07/94	40	1060	36302373	0.036	0.014	0.05	0.003	0.026
20/07/94	40	1061	23678290	0.045	0.013	0.061	0.008	0.037
21/07/94	180	1062	18293000	0.11	0.077	0.301	0.031	0.136
22/07/94	180	1071	23103636	0.97	0.072	0.317	0.034	0.132
25/07/94	180	1084	25639403	0.131	0.063	0.316	0.033	0.14
05/08/94	180	1098	45277384	0.124	0.085	0.321	0.039	0.126
08/08/94	180	1112	29449304	0.122	0.071	0.306	0.038	0.116
09/08/94	180	1114	34562238	0.12	0.077	0.324	0.03	0.131
10/08/94	180	1121	43567944	0.11	0.064	0.33	0.031	0.13
11/08/94	180	1127	19487573	0.111	0.06	0.321	0.027	0.14
12/08/94	180	1135	45643947	0.103	0.079	0.32	0.029	0.138
15/08/94	180	1138	24374874	0.104	0.077	0.31	0.036	0.122
16/08/94	180	1143	10038474	0.99	0.063	0.311	0.041	0.133
17/08/94	180	1152	56733090	0.98	0.069	0.315	0.04	0.124
18/08/94	180	1157	37804939	0.122	0.065	0.309	0.035	0.125
19/08/94	500	1164	39474683	0.118	0.215	1.07	0.123	0.327

19/08/94	500	1165	48464893	0.27	0.203	0.998	0.1	0.308
19/08/94	500	1166	20489575	0.265	0.217	0.89	0.128	0.331
19/08/94	360	1167	34364859	0.209	0.136	0.699	0.079	0.21
19/08/94	360	1168	39846474	0.188	0.133	0.705	0.066	0.232
19/08/94	360	1169	68467584	0.2	0.152	0.716	0.068	0.214
19/08/94	270	1170	47589594	0.157	0.127	0.514	0.042	0.153
19/08/94	270	1171	56565943	0.153	0.13	0.502	0.051	0.172
19/08/94	270	1172	59447474	0.166	0.11	0.478	0.06	0.16
19/08/94	180	1173	39945057	0.129	0.06	0.302	0.034	0.138
19/08/94	180	1174	27399936	0.11	0.08	0.311	0.035	0.127
19/08/94	180	1175	23563340	0.108	0.076	0.297	0.03	0.133
19/08/94	90	1176	17300383	0.081	0.028	0.134	0.022	0.069
19/08/94	90	1177	51846493	0.065	0.021	0.135	0.013	0.086
19/08/94	90	1178	56392987	0.07	0.034	0.118	0.017	0.1
19/08/94	40	1179	14264739	0.032	0.02	0.075	0.005	0.029
19/08/94	40	1180	16356483	0.024	0.013	0.051	0.009	0.018
19/08/94	40	1181	35283744	0.044	0.008	0.06	0.016	0.035
22/08/94	180	1182	58463937	0.113	0.078	0.326	0.033	0.117
23/08/94	180	1183	19374749	0.116	0.083	0.318	0.028	0.132
24/08/94	180	1188	11285733	0.104	0.082	0.315	0.026	0.134
25/08/94	180	1205	49000353	0.109	0.07	0.326	0.037	0.132
29/08/94	180	1210	37383036	0.11	0.077	0.334	0.027	0.129
30/08/94	180	1222	25374037	0.122	0.069	0.327	0.029	0.126
31/08/94	180	1240	20404057	0.126	0.065	0.319	0.035	0.119
01/09/94	180	1250	47468494	0.129	0.079	0.331	0.026	0.141
02/09/94	180	1259	38394048	0.109	0.073	0.329	0.032	0.132
03/09/94	180	1269	35330033	0.12	0.064	0.302	0.026	0.136
05/09/94	180	1278	40047389	0.101	0.06	0.319	0.033	0.119
15/09/94	180	1287	16374800	0.106	0.081	0.326	0.025	0.124
16/09/94	180	1303	53293707	0.104	0.082	0.312	0.034	0.137
19/09/94	180	1318	24000376	0.126	0.075	0.307	0.033	0.109
19/09/94	180	1319	64839023	0.089	0.063	0.3	0.029	0.121
19/09/94	90	1320	44638374	0.06	0.031	0.128	0.018	0.064
19/09/94	90	1321	68492043	0.052	0.02	0.112	0.013	0.079
19/09/94	40	1322	47493944	0.033	0.013	0.069	0.004	0.032
19/09/94	40	1323	39476390	0.035	0.004	0.053	0.001	0.04
20/09/94	180	1327	56489338	0.109	0.073	0.303	0.035	0.126
21/09/94	180	1339	25374930	0.11	0.072	0.322	0.032	0.124
22/09/94	180	1352	60848504	0.12	0.066	0.324	0.036	0.118
26/09/94	180	1359	36474839	0.117	0.069	0.33	0.027	0.13
27/09/94	180	1374	43748933	0.113	0.074	0.316	0.034	0.12
28/09/94	180	1384	28567493	0.123	0.082	0.323	0.037	0.133
29/09/94	180	1398	24986643	0.115	0.08	0.324	0.026	0.127
30/09/94	180	1413	57487333	0.103	0.085	0.3	0.029	0.121
01/10/94	180	1425	38464939	0.12	0.061	0.305	0.034	0.137
03/10/94	180	1430	39575674	0.102	0.063	0.325	0.026	0.137
04/10/94	180	1439	28374644	0.109	0.075	0.32	0.027	0.131
05/10/94	180	1456	46439049	0.113	0.082	0.324	0.037	0.118
06/10/94	180	1475	36449484	0.115	0.059	0.31	0.031	0.124
07/10/94	180	1490	39485943	0.107	0.06	0.317	0.032	0.12
10/10/94	180	1495	40595895	0.98	0.072	0.334	0.041	0.139
11/10/94	180	1509	45463393	0.99	0.078	0.324	0.039	0.127
12/10/94	180	1523	10384738	0.101	0.081	0.325	0.026	0.136
13/10/94	180	1540	40846370	0.105	0.067	0.301	0.031	0.132
14/10/94	180	1550	26527382	0.105	0.074	0.316	0.03	0.123
17/10/94	180	1558	43840400	0.11	0.085	0.329	0.032	0.117
18/10/94	180	1565	36302849	0.125	0.08	0.325	0.039	0.129

19/10/94	500	1579	26379048	0.262	0.237	0.887	0.099	0.302
19/10/94	500	1580	29475694	0.254	0.201	1.01	0.104	0.323
19/10/94	500	1581	59473487	0.275	0.22	0.986	0.117	0.336
19/10/94	360	1582	18483835	0.201	0.156	0.715	0.084	0.228
19/10/94	360	1583	24484474	0.183	0.153	0.71	0.072	0.217
19/10/94	360	1584	30384785	0.196	0.139	0.688	0.075	0.234
19/10/94	270	1585	39498585	0.14	0.115	0.507	0.067	0.177
19/10/94	270	1586	56483004	0.145	0.102	0.512	0.055	0.159
19/10/94	270	1587	39485035	0.159	0.118	0.496	0.054	0.164
19/10/94	180	1588	64948480	0.106	0.07	0.322	0.037	0.129
19/10/94	180	1589	20475944	0.104	0.077	0.296	0.028	0.136
19/10/94	180	1590	49373639	0.114	0.059	0.307	0.04	0.14
19/10/94	90	1591	39475804	0.063	0.018	0.14	0.011	0.095
19/10/94	90	1592	20884749	0.072	0.038	0.131	0.018	0.092
19/10/94	90	1593	36400484	0.069	0.025	0.115	0.02	0.078
19/10/94	40	1594	69483004	0.04	0.014	0.069	0.001	0.041
19/10/94	40	1595	39478044	0.034	0.005	0.043	0.004	0.03
19/10/94	40	1596	70478648	0.027	0.012	0.062	0.001	0.026
20/10/94	180	1597	35839383	0.123	0.079	0.308	0.029	0.133
21/10/94	180	1613	49564933	0.122	0.063	0.311	0.038	0.131
22/10/94	180	1621	67473947	0.109	0.065	0.318	0.032	0.13

Appendix 10 : Field-blank ratio (ISR) variation

Variation in the field-blank ratio (ISR) over the year (Section 5.4). The two-tailed unpaired t-tests are given which determine whether there is a significant difference between means at the 95% confidence level.

Appendix 11 : $C_2H_3Cl_3$ calculation as an example for ng/g calculation

$C_2H_3Cl_3$ calculation as an example of the ng/g calculation (Section 5.5). The slope (response factor) and intercept given are from the calibration plot described in Chapter 5. The calculation uses these values to obtain the field blank concentration X^ from the field blank ratios. The sample concentration was calculated by subtracting the mean field-blank from the ISR (to give the corconc) which was then converted to the ng/g. Each individual result was corrected before calculation of the mean and standard deviation for the sampling occasion. Artefacts (art) refer to chromatographic aberrations (Chapter 3) which prevented data being obtained for that sample.

APPENDIX 10 : Field -blank ratios over all time												
C2H3Cl3			**CCl4**			**C2HCl3**			**C2Cl4**			
Banchory	**Ponsonby**	**Devilla**	**Banchory**	**Ponsonby**	**Devilla**	**Banchory**	**Ponsonby**	**Devilla**	**Banchory**	**Ponsonby**	**Devilla**	
0.000453	0.00152	0.0003	0.00428	0.00973	0.00435	0.00455	0.00471	0.0099	0.00223	0.00023	0.0004	
0.000972	0.00118	0.0009	0.00896	0.0224	0.00779	0.0141	0.0174	0.0016	0.00666	0.00158	0.0003	
0.0009	0.000883	0	0.00657	0.00567	0.0125	0.00567	0.0148	0.0011	0.00733	0.0126	0.0023	
0	0.0009	0.0003	0.0185	0.0185	0.00435	0.0379	0.0379	0.002	0.0126	0	0.0215	
0.00225	0	0.0009	0.00915	0.00915	0.00779	0.00722	0.00722	0.0165	0	0	0	
0.00246	0.00362	0	0.00642	0.00642	0.0125	0.00433	0.00433	0.0111	0	0	0	
0.00356	0	0.0051	0.00316	0.00714	0.00339	0.00975	0.0172	0.0158	0	0.000546	0.0031	
0	0.00341	0.0028	0	0.00932	0.00963	0.00196	0.025	0.0257	0	0.00307	0.0016	
0	0	0.0063	0.00677	0.0124	0.00285	0.00104	0.00617	0.0161	0.00118	0.00158	9E-05	
0	0	0	0	0	0.00637	0	0.0111	0.0028	0	0.000092	0.0148	
0.0671	0.000261	0.007	0	0	0.00445	0	0.0158	0.0114	0	0.0092	0.0105	
0	0.000866	0.0003	0.0367	0	0	0.00985	0.0257	0.0301	0	0.00675	0.0175	
0	0	0.0033	0.101	0.00243	0.0758	0.00306	0.0089	0.0186	0.00113	0	0	
0.0671	0.00683	0.0025	0	0.0146	0.0204	0	0.03	0.0239	0	0	0	
0	0.00476	0.0132	0	0.00372	0.099	0.0081	0.0151	0.0124	0.00088	0	0.0045	
0	0	0.0019	0.00677	0.00184	0.0545	0	0.0131	0.0181	0	0	0	
0	0	0.0008	0.0367	0	0.00687	0	0.00289	0.0249	0	0	0	
0.0671	0.00683	0.0124	0	0	0.00371	0.00985	0	0	0.00113	0	0	
0	0.00118	0.0025	0	0.00278	0.0121	0.0081	0	0.0003	0.00088	0	0.0015	
0.005346			0.012622			0.010966			0.00259			
mean over all C2H3Cl3			mean over all CCl4			mean over all C2HCl3			mean over all C2Cl4			
0.01496			0.021523			0.009913			0.004866			
sd over all C2H3Cl3			sd over all CCl4			sd over all C2HCl3			sd over all C2Cl4			
0.029921			0.043046			0.019826			0.009732			
2sd			2sd			2sd			2sd			
0.011152	0.001697	0.0032	0.012894	0.006637	0.01833	0.006604	0.013543	0.0128	0.001791	0.001876	0.0041	
mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	
0.024911	0.002279	0.004	0.024091	0.006644	0.02728	0.008708	0.010397	0.0096	0.003386	0.003627	0.0067	
sd	sd	sd	sd	sd	sd	sd	sd	sd	sd	sd	sd	
0.049822	0.004558	0.008	0.048182	0.013287	0.05457	0.017416	0.020794	0.0191	0.006772	0.007255	0.0135	
2sd	2sd	2sd	2sd	2sd	2sd	2sd	2sd	2sd	2sd	2sd	2sd	

If two tailed unpaired test shows P(T<=t) greater than 0 then the difference between means
is not significant at the 95% level

Banchory-Ponsonby CCl4
t-Test: Two-Sample Assuming Unequal Var

	Variable 1	Variable 2
Mean	0.012894	0.0066
Variance	0.00058	4E-05
Observatio	19	19
Hypothesiz	0	
df	21	
t Stat	1.091346	
P(T<=t) on	0.143742	
t Critical or	1.720744	
P(T<=t) tw	0.287484	
t Critical tw	2.079614	

Banchory-Devilla CCl4
t-Test: Two-Sample Assuming Unequal Vari

	Variable 1	Variable 2
Mean	0.01289	0.018334
Variance	0.00058	0.000744
Observatio	19	19
Hypothesiz	0	
df	35	
t Stat	-0.6516	
P(T<=t) on	0.25947	
t Critical on	1.68957	
P(T<=t) tw	0.51894	
t Critical tw	2.03011	

Ponsonby-Devilla CCl4
t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	Variable 2
Mean	0.006637	0.018334
Variance	4.41E-05	0.000744
Observa	19	19
Hypothe	0	
df	20	
t Stat	-1.81581	
P(T<=t)	0.042211	
t Critical	1.724718	
P(T<=t)	0.084423	
t Critical	2.085962	

All data combined for CCl4

Banchory-Ponsonby C2H3Cl3
t-Test: Two-Sample Assuming Unequal Var

	Variable 1	Variable 2
Mean	0.011152	0.0017
Variance	0.000621	5E-06
Observatio	19	19
Hypothesiz	0	
df	18	
t Stat	1.647651	
P(T<=t) on	0.058385	
t Critical or	1.734063	
P(T<=t) tw	0.116769	
t Critical tw	2.100924	

Banchory-Devilla C2H3Cl3
t-Test: Two-Sample Assuming Unequal Vari

	Variable 1	Variable 2
Mean	0.01115	0.003189
Variance	0.00062	1.6E-05
Observatio	19	19
Hypothesiz	0	
df	19	
t Stat	1.37577	
P(T<=t) on	0.09245	
t Critical on	1.72913	
P(T<=t) tw	0.18489	
t Critical tw	2.09302	

Ponsonby-Devilla C2H3Cl3
t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	Variable 2
Mean	0.003189	0.001697
Variance	1.6E-05	5.19E-06
Observa	19	19
Hypothe	0	
df	29	
t Stat	1.412399	
P(T<=t)	0.084237	
t Critical	1.699127	
P(T<=t)	0.168474	
t Critical	2.045231	

All data combined for C2H3Cl3

Banchory-Ponsonby C_2HCl_3				Banchory-Devilla C_2HCl_3				Ponsonby-Devilla C_2HCl_3				
t-Test: Two-Sample Assuming Unequal Var				t-Test: Two-Sample Assuming Unequal Vari				t-Test: Two-Sample Assuming Unequal Variances				
	Variable 1	*Variable 2*			*Variable 1*	*Variable 2*			*Variable 1*	*Variable 2*		
Mean	0.006604	0.0135		Mean	0.0066	0.012751		Mean	0.013543	0.012751		
Variance	7.58E-05	0.0001		Variance	7.6E-05	9.13E-05		Varianc	0.000108	9.13E-05		
Observatio	19	19		Observatio	19	19		Observa	19	19		
Hypothesiz	0			Hypothesiz	0			Hypothe	0			
df	35			df	36			df	36			
t Stat	-2.230234			t Stat	-2.0722			t Stat	0.244654			
P(T<=t) on	0.016121			P(T<=t) on	0.02273			P(T<=t)	0.404057			
t Critical or	1.689573			t Critical on	1.6883			t Critical	1.688297			
P(T<=t) tw	0.032242			P(T<=t) tw	0.04547			P(T<=t)	0.808114			
t Critical tw	2.03011			t Critical tw	2.02809			t Critical	2.028091			
All data combined for	as in 8 combinations at 95% level expect one to show significance by chance											
C_2HCl_3												
Banchory-Ponsonby C_2Cl_4				Banchory-Devilla C_2Cl_4				Ponsonby-Devilla C_2Cl_4				
t-Test: Two-Sample Assuming Unequal Var				t-Test: Two-Sample Assuming Unequal Vari				t-Test: Two-Sample Assuming Unequal Variances				
	Variable 1	*Variable 2*			*Variable 1*	*Variable 2*			*Variable 1*	*Variable 2*		
Mean	0.001791	0.0019		Mean	0.00179	0.004104		Mean	0.001876	0.004104		
Variance	1.15E-05	1E-05		Variance	1.1E-05	4.54E-05		Varianc	1.32E-05	4.54E-05		
Observatio	19	19		Observatio	19	19		Observa	19	19		
Hypothesiz	0			Hypothesiz	0			Hypothe	0			
df	36			df	27			df	28			
t Stat	-0.075264			t Stat	-1.3373			t Stat	-1.269			
P(T<=t) on	0.470211			P(T<=t) on	0.09615			P(T<=t)	0.107445			
t Critical or	1.688297			t Critical on	1.70329			t Critical	1.70113			
P(T<=t) tw	0.940421			P(T<=t) tw	0.1923			P(T<=t)	0.21489			
t Critical tw	2.028091			t Critical tw	2.05183			t Critical	2.048409			
Data combined for C_2Cl_4												

APPENDIX 11

C2H3Cl3 calculation for ng/g			-0.011246	intercept	0.000457	slope		
X(conc)	X*X	Y(ratio)	Y*Y	X*Y				
500	250000	0.211	0.044521	105.5	sigmaX	34580		
500	250000	0.232	0.053824	116				
500	250000	0.22	0.0484	110	sigmaX*X	10976000		
360	129600	0.15	0.0225	54				
360	129600	0.147	0.021609	52.92	sigmaY	13.913		
360	129600	0.156	0.024336	56.16				
270	72900	0.112	0.012544	30.24	sigmaY*Y	1.971655		
270	72900	0.106	0.011236	28.62				
270	72900	0.115	0.013225	31.05	sigmaX*Y	4626.92		
180	32400	0.073	0.005329	13.14				
180	32400	0.068	0.004624	12.24	sigmax*x	3858283		
180	32400	0.082	0.006724	14.76				
90	8100	0.022	0.000484	1.98	sigmay*y	0.819443		
90	8100	0.018	0.000324	1.62				
90	8100	0.029	0.000841	2.61	sigmax*y	1763.161		
40	1600	0.01	0.0001	0.4				
40	1600	0.008	0.000064	0.32	sigmadyx2	0.013713		
40	1600	0.013	0.000169	0.52				
180	32400	0.069	0.004761	12.42	t166,95	1.974358		
180	32400	0.079	0.006241	14.22				
90	8100	0.019	0.000361	1.71	syx2	8.26E-05		
90	8100	0.021	0.000441	1.89				
40	1600	0.013	0.000169	0.52	syx	0.009089		
40	1600	0.009	0.000081	0.36		regression standard error		
500	250000	0.21	0.0441	105	tsyx/b	39.26633		
500	250000	0.209	0.043681	104.5				
500	250000	0.222	0.049284	111	c2	0.0004		
360	129600	0.155	0.024025	55.8				
360	129600	0.169	0.028561	60.84				
360	129600	0.142	0.020164	51.12				
270	72900	0.119	0.014161	32.13				
270	72900	0.11	0.0121	29.7				
270	72900	0.127	0.016129	34.29				
180	32400	0.077	0.005929	13.86				
180	32400	0.071	0.005041	12.78				
180	32400	0.06	0.0036	10.8				
90	8100	0.027	0.000729	2.43				
90	8100	0.021	0.000441	1.89				
90	8100	0.03	0.0009	2.7				
40	1600	0.013	0.000169	0.52				
40	1600	0.019	0.000361	0.76				
40	1600	0.007	0.000049	0.28				
180	32400	0.064	0.004096	11.52				
180	32400	0.08	0.0064	14.4				
90	8100	0.035	0.001225	3.15				
90	8100	0.021	0.000441	1.89				
40	1600	0.012	0.000144	0.48				
40	1600	0.01	0.0001	0.4				
500	250000	0.23	0.0529	115				
500	250000	0.225	0.050625	112.5				
500	250000	0.217	0.047089	108.5				
360	129600	0.14	0.0196	50.4				

360	129600	0.145	0.021025	52.2				
360	129600	0.157	0.024649	56.52				
270	72900	0.103	0.010609	27.81				
270	72900	0.116	0.013456	31.32				
270	72900	0.109	0.011881	29.43				
180	32400	0.079	0.006241	14.22				
180	32400	0.071	0.005041	12.78				
180	32400	0.089	0.007921	16.02				
90	8100	0.029	0.000841	2.61				
90	8100	0.025	0.000625	2.25				
90	8100	0.017	0.000289	1.53				
40	1600	0.009	0.000081	0.36				
40	1600	0.01	0.0001	0.4				
40	1600	0.006	0.000036	0.24				
180	32400	0.063	0.003969	11.34				
180	32400	0.082	0.006724	14.76				
90	8100	0.019	0.000361	1.71				
90	8100	0.037	0.001369	3.33				
40	1600	0.013	0.000169	0.52				
40	1600	0.005	0.000025	0.2				
180	32400	0.086	0.007396	15.48				
180	32400	0.069	0.004761	12.42				
90	8100	0.032	0.001024	2.88				
90	8100	0.017	0.000289	1.53				
40	1600	0.012	0.000144	0.48				
40	1600	0.001	0.000001	0.04				
500	250000	0.212	0.044944	106				
500	250000	0.23	0.0529	115				
500	250000	0.245	0.060025	122.5				
360	129600	0.159	0.025281	57.24				
360	129600	0.168	0.028224	60.48				
360	129600	0.137	0.018769	49.32				
270	72900	0.13	0.0169	35.1				
270	72900	0.102	0.010404	27.54				
270	72900	0.118	0.013924	31.86				
180	32400	0.075	0.005625	13.5				
180	32400	0.06	0.0036	10.8				
180	32400	0.053	0.002809	9.54				
90	8100	0.022	0.000484	1.98				
90	8100	0.031	0.000961	2.79				
90	8100	0.025	0.000625	2.25				
40	1600	0.008	0.000064	0.32				
40	1600	0.017	0.000289	0.68				
40	1600	0.001	0.000001	0.04				
180	32400	0.083	0.006889	14.94				
180	32400	0.078	0.006084	14.04				
90	8100	0.03	0.0009	2.7				
90	8100	0.012	0.000144	1.08				
40	1600	0.017	0.000289	0.68				
40	1600	0.01	0.0001	0.4				
180	32400	0.052	0.002704	9.36				
180	32400	0.07	0.0049	12.6				
90	8100	0.029	0.000841	2.61				
90	8100	0.018	0.000324	1.62				
40	1600	0.015	0.000225	0.6				

40	1600	0.003	0.000009	0.12				
500	250000	0.206	0.042436	103				
500	250000	0.223	0.049729	111.5				
500	250000	0.215	0.046225	107.5				
360	129600	0.153	0.023409	55.08				
360	129600	0.145	0.021025	52.2				
360	129600	0.134	0.017956	48.24				
270	72900	0.129	0.016641	34.83				
270	72900	0.111	0.012321	29.97				
270	72900	0.114	0.012996	30.78				
180	32400	0.078	0.006084	14.04				
180	32400	0.064	0.004096	11.52				
180	32400	0.086	0.007396	15.48				
90	8100	0.014	0.000196	1.26				
90	8100	0.027	0.000729	2.43				
90	8100	0.035	0.001225	3.15				
40	1600	0.009	0.000081	0.36				
40	1600	0.014	0.000196	0.56				
40	1600	0.013	0.000169	0.52				
500	250000	0.215	0.046225	107.5				
500	250000	0.203	0.041209	101.5				
500	250000	0.217	0.047089	108.5				
360	129600	0.136	0.018496	48.96				
360	129600	0.133	0.017689	47.88				
360	129600	0.152	0.023104	54.72				
270	72900	0.127	0.016129	34.29				
270	72900	0.13	0.0169	35.1				
270	72900	0.11	0.0121	29.7				
180	32400	0.06	0.0036	10.8				
180	32400	0.08	0.0064	14.4				
180	32400	0.076	0.005776	13.68				
90	8100	0.028	0.000784	2.52				
90	8100	0.021	0.000441	1.89				
90	8100	0.034	0.001156	3.06				
40	1600	0.02	0.0004	0.8				
40	1600	0.013	0.000169	0.52				
40	1600	0.008	0.000064	0.32				
180	32400	0.075	0.005625	13.5				
180	32400	0.063	0.003969	11.34				
90	8100	0.031	0.000961	2.79				
90	8100	0.02	0.0004	1.8				
40	1600	0.013	0.000169	0.52				
40	1600	0.004	0.000016	0.16				
500	250000	0.237	0.056169	118.5				
500	250000	0.201	0.040401	100.5				
500	250000	0.22	0.0484	110				
360	129600	0.156	0.024336	56.16				
360	129600	0.153	0.023409	55.08				
360	129600	0.139	0.019321	50.04				
270	72900	0.115	0.013225	31.05				
270	72900	0.102	0.010404	27.54				
270	72900	0.118	0.013924	31.86				
180	32400	0.07	0.0049	12.6				
180	32400	0.077	0.005929	13.86				
180	32400	0.059	0.003481	10.62				

90	8100	0.018	0.000324	1.62				
90	8100	0.038	0.001444	3.42				
90	8100	0.025	0.000625	2.25				
40	1600	0.014	0.000196	0.56				
40	1600	0.005	0.000025	0.2				
40	1600	0.012	0.000144	0.48				
34580	10976000	13.913	1.971655	4626.92	sigma			

	ratio	concX^	x^	x^*x^	upper95%	low95%	meanbl
bl1	0.000453	25.600372	-180.233	32483.92	65.08377	-14.0271	26.168206
bl2	0.000972	26.736039	-179.097	32075.84	66.21783	-12.8889	sd
bl3	art	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0.8030381
	ratio	conc	corconc	ng/g	mean field blank corrected		
dat1	art	#VALUE!	#VALUE!	#VALUE!	21.93922		
dat2	0.00469	34.871707	8.703501	30.43182	sd		
dat3	0.00247	30.013939	3.845733	13.44662	12.01035		
Banchory 10m 1992 needles							
25/03/93 collection							

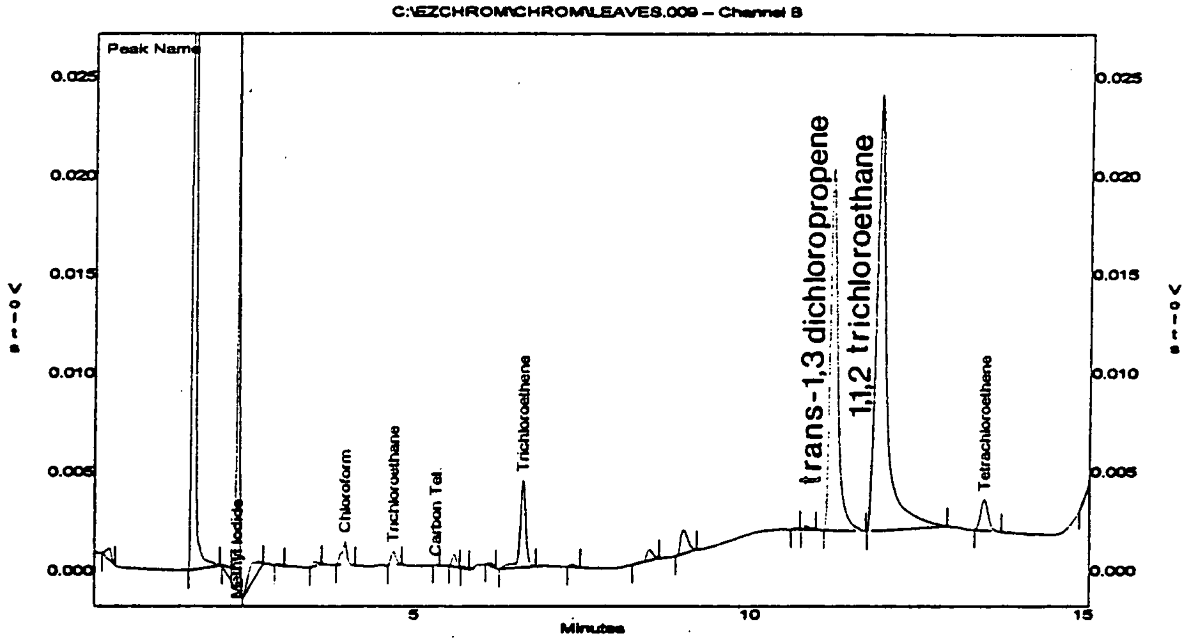
Appendix 12 : Example of trial 'Optic' injector results

Operating conditions for the 'Optic' injector trials :

Column :	BP1, (25m x 0.32mm ID, 1μm film). Temperature ramp : 35°C 8 min hold, 5°C/min to 45°C, 5 min hold, 25°C/min to 120°C, 1 min hold.
Carrier :	H_2 @ 3.0 psi
Injector :	140°C, 30-60 mins desorption time, split flow @ 20ml/min
Detector :	ECD @ 300°C

University of Edinburgh Tree Contamination

File : C:\EZCHROM\CHROM\LEAVES.009
Method : C:\EZCHROM\METHODS\LEAVES.MET
Sample ID : 30ft Devilla 1993 Field 06109
Acquired : Feb 09, 1994 13:07:33



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Channel B Results

          Time         Compound                 'Area
       ----------   ---------------------    ----------
             2.34   Methyl Iodide                 68217
             3.95   Chloroform                     6572
             4.68   Trichloroethane                3680
             5.32   Carbon Tet.                     136
             6.62   Trichloroethene               26178
            13.54   Tetrachloroethene             12237
```

Courses attended

Gas chromatography seminar, J&W chromatography, Stirling, November 1991.
British Nuclear Fuels plc. Christmas lectures, December 1991.
British Nuclear Fuels plc. Student Research Forum, 1991 and 1992.
Environmental Chemistry (Year 4) lectures (University of Edinburgh) 1991-1992.
Faraday Lectures, Dundee, February 1992.
Departmental German course taken and passed, March 1992.
Conference : Society of Environmental Geochemistry and Health, Edinburgh, April 1992.
Seminar : Capillary columns, Glasgow, April 1992.
Environmental Chemistry project talks (University of Edinburgh) 1994.
Chemistry Society lectures (University of Edinburgh) 1991-1994.
Symposium : Royal Society of Chemistry, Analytical Division. Alternatives to chemical solvents restricted by the Montreal Protocol, London, February 1995.

Reports and presentations

Quarterly reports for British Nuclear Fuels plc. 1991-1994.
Presentation to Environmental Section of Department (University of Edinburgh), March 1992.
First year report and viva (University of Edinburgh) May 1992.
British Nuclear Fuels plc. Student conference, December 1992. Poster award.
Conference : Euroanalysis VIII, Edinburgh, September 1993. Poster presentation.
First conference on naturally-produced organohalogens, Delft, The Netherlands, September, 1993. Poster presentation.
Presentation : 'Solvent abuse and trees : Can the environment cope?' British Nuclear Fuels plc. Student Research Forum, April 1994 .
RSC Autumn conference, Glasgow, September 1994. Poster award.
SETAC conference, Sheffield, September 1994. Poster and presentation award.

Published paper

Brown, R.H.A., Cape, J.N. and Farmer, J.G. (1995) Chlorinated C_1- and C_2-hydrocarbons in the needles of Scots Pine (*Pinus sylvestris L.*) in three forests of Northern Britain. Naturally-Produced Organohalogens. Environment and Chemistry 1, 397-401. Kluwer Academic Publishers.