The Distribution and Fluxes of Trichloroacetic Acid in a Sitka Spruce Forest

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Abstract

Trichloroacetic acid (TCAA: CCl₃COOH) is a known phytotoxic chemical and its presence in tree foliage has led to suggestions that it is a stressor in forest decline. However, the source(s) of TCAA to remote forest ecosystems remains the subject of much debate. One possible source of TCAA is the atmospheric oxidation of anthropogenic chlorinated solvents such as 1,1,1-trichlorethane and tetrachlorethene, but it has also been suggested that TCAA may be produced naturally in soils by the action of chloroperoxidase enzymes on organic matter and chloride substrates. The relative magnitudes of these natural and anthropogenic fluxes and pathways of TCAA through the forest system are not yet established. The work reported in this thesis represents the first detailed measurements of TCAA in the UK and was instigated with the aim of helping to resolve these uncertainties.

Two analytical methods were used in this work to determine TCAA concentrations in natural matrices, both utilising the property of thermal decarboxylation of TCAA to CHCl₃. Initially, a novel modified steam distillation method was developed for the preliminary analyses of TCAA in conifer needles. Subsequently a more direct method using headspace gas-chromatography (HSGC) was developed to permit the automated analysis of various environmental matrices.

Detailed TCAA data are presented from an 18 month period of intensive field sampling at two elevations (602 m and 325 m a.s.l.) in a remote upland Sitka spruce (Picea sitchensis) forest in southern Scotland. Concentrations of TCAA in different year classes of spruce needles, and in air, rain water, cloud water and forest soil were measured concurrently at sites with a wet deposition gradient to investigate the route of atmospheric input to forest.

The observations of greater concentrations of TCAA in the needles at higher elevation (mean concentration of 1998 year class: 38 and 28 ng g⁻¹ dry weight at the upper and lower elevations, respectively), a trend towards accumulation of TCAA in older needles, and seasonal variations are all consistent with the hypothesis that the TCAA concentration may be controlled by metabolic processes in the needles.
Higher concentrations of TCAA in cloud water (median: 0.94 ppb) than rain water (median: 0.87 ppb) with ratios as high as 7:1 at some times have been measured. No seasonal variation of TCAA in wet input to the forest has been observed. The sum of gaseous and particle-bound concentrations of TCAA in air is low (<100 pg m$^{-3}$). Concentrations of TCAA in forest soil are extremely variable (5 to 400 ng g$^{-1}$ fresh weight) with location and time, and the origin of some very high, but repeatable, measurements (>200 ng g$^{-1}$ fresh weight), which cannot be explained by atmospheric input only, has not been identified. The percentage of TCAA, which can be extracted from the soil with water, is shown to be <10%, suggesting that TCAA is adsorbed or bound to the soil matrix. No significant relationship between soil TCAA concentrations and total organic matter content, water content or pH has been observed. A zero-dimensional mass balance calculation based on measurements at the upper forested site has identified a net source of TCAA in the catchment, suggesting that a natural formation of TCAA in the soil is occurring. However, the nature of the formation process is not, at this stage, identified.

The results from parallel greenhouse experiments, in which solutions with concentrations of 0, 10 and 100 ppb TCAA were applied, in a complete two factorial design, to 120 Sitka spruce seedlings via foliage only or soil only, indicate that an above-ground route of TCAA input, possibly via the stems, is important and may be more effective at increasing the needle TCAA concentrations than the soil route.
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Chapter 1

Introduction

1.1 Background

The study of pollutants in the environment has become more widespread as the limits of detection of instrumentation have become more sensitive so that compounds present at trace levels in the environment can now be monitored. As the limits of detection improve substances may be detected that have the potential to cause damage to living systems, but for which so far direct evidence is lacking. Knowledge of the sources, fate, concentrations and behaviour of trace pollutants is required to elucidate the effect that particular compounds may have on different environmental compartments and their persistence therein. The most obvious environmental pollution has been of a gross nature and the effects of entities such as atmospheric acidity have been clearly observed and well studied. Sulphur dioxide at levels of several hundred micrograms per cubic meter is known to be acutely phytotoxic (Legge et al. 1980) and high ozone concentrations have been associated with chlorotic mottling and needle loss in conifers (Dochinger & Seliskar 1970). The trace pollutants that are now studied in addition to those above are more insidious, their presence can only be detected with very sensitive equipment and the potential damage that they wreak can lie undetected for years or even decades.

One such class of compounds is the chloroacetic acids, which includes monochloro- (MCAA), dichloro- (DCAA) and trichloroacetic acid (TCAA). The key property that makes this family of chemicals an environmentally significant group to study is their phytotoxicity. Conifers in mountain forests of the industrialised countries of the Northern Hemisphere have suffered extensive, premature needle loss for more than a decade. Chlorosis and necrotic spots on needles and leaves of the sun exposed canopy, reduced size and altered shapes of leaves, and degeneration of fine roots and mycorrhiza are other typical symptoms (Schütt & Cowling 1985). The widespread forest decline experienced throughout Europe and North America over the last decade
has been suggested to be caused by atmospheric pollutants, and amongst the chemicals implicated were the chloroacetic acids, although it is now the most commonly accepted hypothesis that forest decline is the result of the combined action of several atmospheric stress factors (Frank et al. 1991). Findings that forest decline can occur in areas, which until recently were considered as ‘clean-air’ regions and that similar needle loss symptoms were spreading throughout Europe and North America suggested that anthropogenic air pollutants or secondary phytotoxic products derived from them were involved. Ozone, nitrogen oxides and sulphur dioxide are the only compounds with sufficient data for a causal relationship to be proposed. In this study TCAA is of particular interest with only small reference made to the other chloroacetic acids. However, many of the principles and possibly the approaches used could also be applied to the other members of the family.

TCAA was commercially available as its sodium salt or in ester and amide derivatives as a herbicide for the treatment of perennial grasses and monocotyledonous weeds from the late 1940s onwards (Ashton & Crafts 1973). The only recent use of TCAA as a herbicide was in Ontario where TCAA was approved for use in controlling quack grass, annula grasses and conifers in barley, oats and red beet crops (Guide to Weed Control 1997). Likewise MCAA has anilide derivatives which are employed for weed control in products such as Alachlor, Propachlor, Metolachlor and Metazachlor (Frank et al. 1994). Experiments performed with weeds and crop plant species have shown that TCAA exposure at high levels may cause growth inhibition, leaf necrosis, morphological effects and changes in the properties of the surface wax (Ashton & Crafts 1973). This not only included weeds but also woody plant species (Barrons & Hummer 1951; Åberg 1982). Inhibition of pantothenate synthesis, the precursor of coenzyme A, has been suggested as the biochemical basis of its phytotoxicity (Hilton et al. 1959). This poor selectivity of TCAA as a herbicide led to it being banned from use in 1970’s in Europe (Frank et al. 1992). However, it was not until after this ban was imposed that its presence in the environment was noticed and its potential impact was recognised, especially as its source was not known.
Long after TCAA had been discontinued from use as a herbicide its presence is still being detected in conifer needles and precipitation at ng g$^{-1}$ and ppt levels in remote areas such as Northern Finland (Frank et al. 1994; Norokorpi & Frank 1995). Though TCAA is still used as an etching/pickling agent in surface treatment of metals, as a swelling agent and solvent in the plastics industry and as an auxiliary in textile finishing it is thought unlikely that these would be direct sources to such remote areas. Even with accidental releases of TCAA, long distance transport is thought to be unlikely due to the scavenging effect of rain on such a hydrophilic compound. All this points to the presence of indirect sources of TCAA created from certain precursors readily available in the environment.

The first suggestion for the identity of these TCAA precursors was C$_2$-chlorocarbons, which were widely used in industry for degreasing metals and dry cleaning textiles. This group includes 1,1,1-trichloroethane, trichloroethene and tetrachloroethene (also called perchloroethylene or PER). Mechanisms have been proposed for the photooxidation of PER to trichloroacetyl chloride in the atmosphere followed by aqueous oxidation to TCAA (Figure 1.1) and the photooxidation of 1,1,1-trichloroethane to chloral with subsequent hydrolysis to TCAA (Figure 1.2). Trichloroethene has also been shown to be a source of DCAA. Despite the feasibility of these reactions the TCAA produced in both schemes is only the minor product with low yields (5-15% Figure 1.1; 1-5% Figure 1.2) (Sidebottom & Franklin 1996; Franklin 1995) and may not account for the concentrations measured in the environment. This is a focus of much continued debate amongst the scientific community (Juuti & Hoekstra 1998; Frank et al. 1999).
Figure 1.1; The proposed mechanism for the conversion of tetrachloroethene to TCAA

Figure 1.2; The proposed mechanism for the conversion of 1,1,1-trichloroethane to TCAA
The proposed formation processes seem to support the theory that TCAA found in remote areas at trace levels is formed from the solvent precursors mentioned previously. The presence of the precursors in the troposphere is unquestionable with estimated global releases of 295,000 tonnes tetrachloroethene in 1992 (McCulloch & Midgley 1996), and their long lifetimes in the atmosphere meaning long distance transport to 'clean' remote areas would be possible, followed by conversion to TCAA, atmospheric deposition and uptake by trees.

However, the idea that TCAA is solely formed from these precursors is open to considerable question. The reason for doubt is that work by Boren et al. (1994), Grimvall (1995) and Sidebottom & Franklin (1996) questioned whether the accepted pathway for the formation of TCAA from PER was actually valid. The relative importance of the reaction of the chlorine radical with PER was reported to depend not only on the ratio of the rate constants for attack by the OH and Cl radical, but also on their relative atmospheric concentrations. It was concluded by Rudolph et al. (1996) that the mean chlorine atom concentration may have been close to zero or at most 500 molecules cm$^{-3}$, which was in agreement with Aucott (1996). The work of Grimvall (1995) found that TCAA concentrations in snow and precipitation in Arctic and sub-Arctic regions were very similar to those found in Antarctica. This was not expected as there were six-fold larger emissions of possible precursors of TCAA in the Northern Hemisphere, which led to the expectation of significantly lower concentrations in the Southern Hemisphere measurements (Sidebottom & Franklin 1996). These theoretical and experimental findings led Sidebottom & Franklin (1996) to conclude that the main source of TCAA in precipitation was not PER. Additional measurements (von Sydow et al. 1999) have supported this conclusion as TCAA was found to be present in glacier ice samples from Marmaglaciären in Northern Sweden (dated to an age of 180 to 200 years) from Storglaciären (dated older than 500 years), in firm from Antarctica (from the beginning of the 20th century) and in glacier ice samples from Monte Rosa in Switzerland (dated to around 1900). These samples were from a time before the mass production of
chlorinated solvents and so it was unlikely that this TCAA originated from anthropogenic sources. However, Sidebottom & Franklin (1996) conceded that it was not possible to exclude a minor contribution of PER to atmospheric TCAA.

It is not only evidence from snow and ice samples that suggested a possible natural production of TCAA, but also findings from research into soil samples. Asplund \textit{et al.} (1991, 1993 & 1994) were successful in isolating a soil extract with halogenating potential. The ability of humic substances in water to produce TCAA has been shown by Haiber \textit{et al.} (1996). This chlorination potential was thought to occur through the chlorination of the phenolic rings present in humics. Production of TCAA through enzyme activity on short chain aliphatics such as acetic acid in soil was demonstrated with the optimum reaction occurring at pH 3-4. However, humic acid with and without chloroperoxidase enzymes (CPO) both led to TCAA formation, with reduced yields without the enzyme. Recent studies by Hoekstra & De Leer (1995a) found that the previously isolated fungus \textit{Caldariomyces fumago} (De Jong \textit{et al.} 1994) was able to synthesise hypochlorite from inorganic chloride due to oxidation by hydrogen peroxide, which in turn could chlorinate organic materials. The CPO enzymes responsible have been shown to produce TCAA as well as other chlorinated organics such as chloroform and DCAA in Dutch nature reserves, probably from enzymatic chlorination of organic matter in the top layer of soil. The activity of CPO has been shown to be greater at lower pH, so it was thought that the acidic soils found in coniferous forests could lead to a greater production of chlorinated species such as TCAA. Hoekstra \textit{et al.} (1999b) questioned whether TCAA levels found in pine needles could be accounted for solely by the uptake of atmospheric degradation products, and suggested that TCAA naturally produced in soils was taken up by the roots of trees and stored in their needles.

There is therefore some evidence that TCAA can be produced naturally in soil. However, so far there are no studies demonstrating this unequivocally in the field, as some laboratory studies used conditions which were unlikely to be found in the natural
environment. It is not possible to say at this stage if this natural process could account for the needle concentrations measured, so the concentrations may prove to result from a combination of in situ production and input from atmospheric photooxidation of TCAA precursors, of which the relative contributions from each are yet to be elucidated. If the reservoir of TCAA in the soil is large, then the contribution of the wet input is likely to be negligible, but if the natural production is small then it is the concentration of TCAA in, and amount of, wet deposition that is the dominant component. The stability and lifetime of TCAA in the soil is also an important factor. If the TCAA produced is quickly degraded then the soil reservoir is likely to be small, thus limiting the TCAA available for uptake by the trees. The half life of TCAA quoted by Hoekstra et al. (1999a) (determined by Worthing & Hance 1991) was 15 to 90 days. This large range in half life is dependent on the soil type. Very few measurements of TCAA half-life in soil exist in the literature at environmental concentrations and it is unlikely that one value is appropriate for all soil types, climates and countries. Half-life determinations must probably be made at each specific site to determine an appropriate value. It is unclear whether the measured loss of TCAA in soil is actually due to the physical binding of TCAA to humic acids or whether it is genuine decomposition, and so these questions must be answered before a reliable understanding of the TCAA cycle in the environment can be produced.

The uptake pathways of TCAA into needles have been studied by Sutinen et al. (1995 & 1997), where TCAA was found to be taken up both by root and needle pathways. Translocation of TCAA from the soil occurs via the transpiration system and TCAA moves along concentration gradients to the needles. Additionally a water film mechanism was suggested, where molecular films were formed from the deposition of deliquescent salts, which could extend into the sub-stomatal cavities through the open stomata so allowing the transportation of anions such as trichloroacetate across concentration gradients (Burkhardt & Eiden 1994). It is uncertain whether this is possible as Schönherr & Bukorac (1972) showed that a liquid film was unlikely to penetrate stomata when
open, as it is difficult for simple ionic species to pass through such a hydrophobic surface even through the stomata. The disintegration of the wax layer was thought to lead to greater transpiration due to uncontrolled water loss and therefore greater TCAA uptake from the root pathway. The wax layer was also seen as a barrier to the transport of ions through the leaf surface so its removal may make direct uptake easier. It was also noted that up to a certain TCAA concentration there appeared to be a metabolic mechanism for the removal of TCAA, until at a critical point the metabolism became overloaded and TCAA concentrations increased rapidly. The foliar route that Sutinen et al. (1995 & 1997) found to exist has not been examined in depth, but it was suggested that 80% of the applied dose remained adsorbed on the needle surfaces and that it was easily removed by rinsing with water. This still suggested that the remaining 20% was somehow taken up through the foliage, which has so far not been explained and may be significant.

It was suggested that TCAA may be partly decarboxylated to CHCl₃ within the needles (Frank 1991; Plümacher 1995) and that the half life of TCAA within spruce needles was 10 days (Frank 1991), but it is not known how this varies from species to species or throughout the year. Therefore TCAA concentrations in needles presumably reflect a steady state between uptake and removal mechanisms. It was also suggested that the uptake of chlorinated solvents into the cuticle of needles could occur (Frank et al. 1992; Plümacher 1995) and that the subsequent enzymatic detoxification by P450 monooxygenases (Frank et al. 1992; Plümacher & Schröder 1994; Plümacher 1995) may lead to TCAA formation akin to the mechanism seen in the livers of rats and humans (Christensen et al. 1988; Køppen et al. 1988). There is little evidence for this and work by Brown et al. (1999) suggested there was no evidence for an accumulation of 1,1,1-trichloroethane, CCl₄, trichloroethene and PER with year class, which would not support the hypothesis of increased TCAA concentrations with needle age. Definitive proof of the route of uptake and formation of TCAA in trees is so far missing. The proposed cycling of TCAA and CHCl₃ in the environment is shown schematically in Figure 1.3 (Hoekstra & De Leer 1995a).
A literature summary of the analytical methodologies used to determine TCAA is presented by Terreni et al. (1995). One standard methodology has not been established for TCAA analysis, but commonly derivatisation of TCAA and analysis by GC is used. Derivatisation is required to produce a compound sufficiently volatile for analysis by GC. Derivatisation of TCAA to its methyl ester using diazomethane is most frequently used (Frank et al. 1990), but this can also be performed using acidified methanol (Reimann et al. 1996). Conversion of TCAA to difluoroanilide (Ozawa & Tsukioka 1990) and pentafluorobenzyl (Sinkkonen et al. 1998) derivatives is also used. An alternative to derivatisation is the analysis of CHCl₃, the decarboxylation product of TCAA, by headspace gas-chromatography as discussed in Chapter 3. Liquid chromatography can also be used to determine TCAA, but the methods are often not sensitive enough to determine TCAA at the ng g⁻¹ level.
TCAA has been determined in many different environmental matrices as previously mentioned. Most of the sites studied have been in Northern and Central Europe with no previous measurements made in the British Isles. The most common matrices measured for TCAA are conifer needles and precipitation. Frank et al. (1990 & 1991) have determined that a seasonal pattern in the needle TCAA concentrations clearly exists in Germany, with the maximum occurring during late summer (August). The evidence of a seasonal maximum supports the hypothesis that photooxidation reactions in the atmosphere lead to the highest TCAA concentrations, which coincide with the highest solar radiation in summer. Similarly some data presented for the concentrations of TCAA in precipitation also suggest a maximum in August (see Chapter 6) and so a link between atmospheric chlorinated solvent conversion and TCAA has been made. However, could the idea of a maximum needle concentration in summer also be explained by an increased soil production rate or would an increase in temperature lead to a decrease in needle TCAA due to increased degradation? Similarly would a correction for the volume of rain collected negate the apparent summer maximum in TCAA, when precipitation is less frequent? A summer maximum concentration in rain may be caused by the occurrence of rain events with small volumes but high TCAA concentrations. Is this seasonal pattern of wet input appropriate for other wetter climates such as Scotland?

The determination of TCAA in the gas-phase has been very much ignored so far, with only one small study by Frank et al. (1995), so would monitoring of gas-phase TCAA concentrations increase the understanding of the overall TCAA cycle? In Table 1.1 the concentrations of TCAA determined in various environmental compartments by various researchers have been collated (Juuti 1997). The range of TCAA concentrations found and the number of analyses are shown. This piecemeal approach of combining studies from different countries and climates, perhaps using different analytical techniques shows the need for an integrated study of TCAA at one location with measurements of concentrations in as many compartments as possible. Currently the literature reports results with a large spread from the mean, so if greater precision could be achieved through consistent methods of analysis and reporting of
results (year class, dry weight or fresh weight etc.) at a single site this would lead to more statistically significant trends and more meaningful conclusions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TCAA concentration</th>
<th>Units</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0.03 - 0.3</td>
<td>ng m⁻³</td>
<td>21</td>
</tr>
<tr>
<td>Rain water</td>
<td>&lt;0.01 - 20</td>
<td>µg l⁻¹</td>
<td>350</td>
</tr>
<tr>
<td>Snow</td>
<td>0.1 - 0.4</td>
<td>µg l⁻¹</td>
<td>2</td>
</tr>
<tr>
<td>Surface water</td>
<td>0.03 - 0.6</td>
<td>µg l⁻¹</td>
<td>78</td>
</tr>
<tr>
<td>Ground water</td>
<td>0.02 - 0.9</td>
<td>µg l⁻¹</td>
<td>110</td>
</tr>
<tr>
<td>Soil water</td>
<td>0.2 - 2.8</td>
<td>µg l⁻¹</td>
<td>40</td>
</tr>
<tr>
<td>Soil</td>
<td>0.1 - 380</td>
<td>aµg kg⁻¹</td>
<td>25</td>
</tr>
<tr>
<td>Leaves</td>
<td>&lt;1 - 44</td>
<td>µg kg⁻¹</td>
<td>9</td>
</tr>
<tr>
<td>Needles</td>
<td>0.6 - 178</td>
<td>µg kg⁻¹</td>
<td>1600</td>
</tr>
</tbody>
</table>

Table 1.1; TCAA concentrations in various environmental compartments

(n = nos. measurements; a = on dry weight basis)

The aim of this study was to determine TCAA in as many environmental compartments as possible concurrently at a single location in Great Britain. This was to provide both absolute concentrations and the relative temporal trends for comparison to those measured and observed in Central Europe. The choice of a site with increased wet deposition with height was to help to elucidate the method of uptake of TCAA into trees. A fully controlled growing and dosing experiment was to provide complementary information on the uptake pathways of TCAA into needles by measuring changes in TCAA concentrations, but the observation of the more subtle physiological effects on the trees themselves was studied along with the trees' ability to detoxify the xenobiotics over periods of low or no exposure, but also low metabolic activity. The relatively new research area of TCAA in soil is investigated more fully and an attempt at identifying the associations in soil and the method of TCAA production is made. A comparison of analytical accuracy is made using two different analytical methods in order to validate their use and the procedure of modified standard addition calibration outlined by Plümacher & Renner (1993). Using
this integrated approach a mass balance calculation similar to Hoekstra et al. (1999a) is made for this particular site, modelled using consistent data produced during this research, to investigate the importance of different compartments as sources, sinks or removal processes of TCAA.
Chapter 2

The development of a novel method for the analysis of trichloroacetic acid; a modified Nielson-Kryger steam (MoNKS) distillation

2.1 Introduction

The analysis of trichloroacetic acid (TCAA) in the environment requires selective, reliable and sensitive analytical methodologies, robust enough to cope with the various sample matrices which are to be investigated. There are many different methodologies available for the analysis of TCAA, which are of varying usefulness for trace analysis. A colorimetric determination of the pyridinium salt of the decarboxylation product of TCAA, chloroform (CHCl₃), is the oldest monitoring method, still in use for detecting mg l⁻¹ levels in the body fluids of workers in the solvent industry (Fujiwara 1916). Ion chromatography (IC) has been used as a more sensitive method of detection of MCAA, DCAA and TCAA in precipitation (Itoh 1989; Müller et al. 1974; Fuchs & Bächmann 1987; Bächmann et al. 1989). Though the existence of haloacetic acids in their dissociated forms at natural pHs make ion chromatography the most direct and applicable method, it is known that organic acids may interfere with the analysis of TCAA in the μg l⁻¹ range (Frank et al. 1990). Investigation into the applicability of IC method during this research has also found that the sensitivity is insufficient for the measurement of TCAA at ng g⁻¹ or sub-ppb concentrations.

The high resolving power and high sensitivity of detection make gas-chromatography (GC) the method of choice for the trace analysis of TCAA. However, the involatility of TCAA makes direct analysis by GC impossible and requires involved sample pretreatment. Various chemical derivatisations of TCAA have been used in the literature (Frank et al. 1990; Ozawa and Tsukioka 1990; Frank et al. 1995) followed by the
analysis of the products by GC with electron-capture detector (ECD) or mass spectrometry (MS). These methodologies have become standard practice for the simultaneous trace detection of the range of haloacetic acids. Despite this, the derivatisation methods, as the group might be referred to, possess several integral drawbacks. Firstly the multi-step nature of the methods provide many opportunities for reduced efficiency, since the removal of TCAA from its sample matrix is dependent on its transfer to solution. Various pre-treatments such as grinding and ultrasonication are used to optimise its transfer, but if bound tightly to a matrix the TCAA might not be removed. Secondly, the time consuming nature of the method is more suited to a large, well established research group where, for example, fresh distillation of ether and preparation of derivatisation solutions might become routine. This makes access to this area of research more difficult for smaller groups with limited resources. Finally there are many safety hazards associated with these methods, particularly the handling and use of diazomethane as a derivatisation agent in inexperienced hands, due to its toxicity and potential explosiveness. The obvious advantage of the derivatisation methods over other techniques is the ability to simultaneously determine the range of haloacetic acids and the absolute detection of the derivatised products by mass spectrometry. It has recently been reported that the derivatives of some HAAs, particularly the diazomethane and pentafluorobenzyl (PFB) esters are unstable and so not suitable for analysis by derivatisation. The PFB ester of TCAA was reported to be unstable (Sinkkonen et al. 1998) and the methyl derivative of brominated HAAs were said to suffer from photo-promoted side reactions in white light (Urbanski 2000).

The other commonly used method of analysis is the thermal decarboxylation of TCAA to CHCl₃ and its analysis by headspace gas-chromatography (HSGC). This is a more direct technique than the derivatisation methods as the sample may be weighed directly into a closed vial and then subsequently analysed, with no chance of contamination or loss of analyte. The disadvantage of HSGC is the possible interferences to the method by background chloroform naturally present in the sample. This technique is discussed fully in Chapter 3, where the development of and
results from HSGC analysis of TCAA is presented. Though automatic HSGC instrumentation is specialised and expensive, a manual version of the method may be performed using simple equipment. This manual method is also labour intensive and the development of this technique for analysis of TCAA led to the search for a more effective complementary analytical technique.

The complementary technique that was developed was based on a version of a steam distillation called a Modified Nielson-Kryger steam (MoNKS) distillation as outlined by Veith & Kiwus (1977). The theory, development and results from this method are outlined below. The aim of the development of a MoNKS distillation was to validate a more reliable technique for the analysis of TCAA in needles by which to investigate TCAA in a forest ecosystem. The manual HSGC technique was developed in competition to MoNKS distillation, but progress had been slow and had shown significant problems (Hansen 1997). A new methodology was required that could be performed without the need for expensive equipment, which could also give a fairly high throughput of samples.

2.1.1 Background to method development

The principle on which the HSGC technique was based was the property of TCAA to undergo thermal decarboxylation on heating, to evolve a volatile product, chloroform (CHCl₃) as shown in Equation 2.1.

\[
\text{Cl}_3\text{C COOH}_{(aq)} \rightarrow \text{CHCl}_3_{(g)} + \text{CO}_2_{(g)}
\]  

Equation 2.1

The reaction proceeds with only mild heating, yielding 1 mole of CHCl₃ per mole of TCAA. The standard use of this reaction in a closed system i.e. a vial should be equally possible in an open system i.e. a distillation. The use of a distillation process which could allow the heating of a solution containing TCAA, whether in a solid matrix slurried in water or in an aqueous solution such as rainwater, whilst still collecting the evolved CHCl₃ would enable the determination of the TCAA concentration in the sample by the decarboxylation reaction. The recycling of the
solution encountered in a distillation process could promote more efficient and complete conversion of TCAA over a pre-determined time period. If the evolved CHCl₃ could be trapped into a known, small volume of solvent then a concentration factor could be achieved and the subsequent solution would be ideal for analysis by gas-chromatography using an ECD, which is very sensitive to electronegative compounds, especially chlorinated compounds. An old method called a MoNKS distillation which had previously been used for the pre-concentration and analysis of volatile compounds in environmental matrices, such as hexachlorobenzene in fish tissue, was a suitable technique for TCAA determination after some modification.

2.1.2 Theory of MoNKS distillation

The steam distillation is one of the oldest methods of separating chemicals on the basis of differences in vapour pressures over water. Although steam distillation was commonly used for flavour and drug analysis (Nielson & Kryger 1969) the large surface area of the glassware and the low collection efficiency of conventional apparatus prevented the use of this technique for the analysis of trace amounts of less volatile organic compounds. Because exhaustive solvent-extraction techniques removed lipids, waxes and related natural products as well as the trace contaminants, extensive chromatographic separations were necessary before the extracts could be analysed for the trace components. Veith & Kiwus (1977) developed a modified Nielson-Kryger type steam distillation apparatus that provided both exhaustive distillation of pesticides and industrial chemicals from water, sediments and tissue and the simultaneous extraction of the distillate into a small volume of organic solvent (Figure 2.1). The solvent extract was generally suitable for direct analysis by GC without prior concentration and clean up.

In applying this relatively old technique to the analysis of TCAA the property of thermal decarboxylation was utilised, with the assumption that TCAA would be totally and reproducibly converted to CHCl₃ by the reflux of TCAA in solution. The samples (if solid) were slurried with a known volume of water and transferred to a round-bottom flask (RBF), which was then attached to the glass joint beneath the
Figure 2.1; A diagram of MoNKS distillation apparatus
apparatus. Due to the volatility of CHCl₃ a Liebig condenser was attached to the top of the apparatus to stop any erroneous losses from the MoNKS distillation. The temperature of the RBF was raised using a heating mantle, which promoted the formation of the CHCl₃ that passed in the steam through the inner tube and condensed on the inside walls of the cooling jacket. The condensate dripped downwards and through a lower density solvent, in this case hexane, which had a high affinity for CHCl₃ into which it partitioned. The water passed into the overflow tube, which served as a solvent trap and permitted only the water to drip back into the RBF. After the optimum extraction time the apparatus was cooled and the extract was removed through the stopcock and stored. Subsequent GC-ECD analysis determined the concentration of CHCl₃ in the hexane extracts, which was related to the amount of TCAA present in the sample.

Certain assumptions and considerations have to be taken into account for this method to be useful for TCAA measurements. The most fundamental correction that must be made is that the method determines the total CHCl₃ present in the sample, which includes any contribution from TCAA after decarboxylation and any background CHCl₃ present on the solid sample or in the solution matrix. Hence blanks correcting for CHCl₃ in water and in hexane, and for TCAA originally present in the water must be subtracted and/ or minimised. To achieve this the sample solutions were degassed using Oxygen-Free-Nitrogen (OFN) prior to MoNKS distillation, which had the dual effect of removing any CHCl₃ adsorbed to the solid sample and any present in the water used. The optimisation and effectiveness of this procedure is discussed in Section 2.4.3. It was retrospectively discovered that TCAA is generally present in ng ml⁻¹ quantities in most water supplies. Even HPLC grade water contains TCAA at certain levels. This cannot be removed easily and therefore made some contribution to the total CHCl₃ determined in the samples. The concentrations are generally consistent when using the same supply of water and so can easily be corrected for (see Section 2.3.3). In order to enhance the sensitivity and detection limit of the method the blank contribution from water must be minimised. This took the form of using a constantly low volume of water in which to slurry solid samples.
After use the apparatus was washed with methanol and stored in an oven at 200°C until the next analyses. This was designed to ensure that no volatile compounds were present at the start of a new distillation.

This distillation procedure facilitates a considerable concentration step, for example if 200 ml of rainwater was extracted into 5 ml hexane this represents a 40 fold concentration step. If a solid sample is to be analysed the TCAA from a large mass of sample, depending on availability, could be concentrated into a 5 ml extract. For these assumptions to hold the volume of hexane used must be accurately and reproducibly delivered. To achieve this ‘A’ grade pipettes of high precision were used to measure the hexane volume. The final assumption is that during the MoNKS distillation no loss of hexane or CHCl₃ from the system is experienced and the trapped CHCl₃ is evenly distributed within the volume of hexane. This is a fair assumption as both the action of reflux and the process of removal of the hexane from the apparatus by their nature shake and mix the hexane layer. These assumptions enable the collection of the majority of the extract, which exhibits a CHCl₃ concentration representative of the total volume. If this were not true then a quantitative collection of the hexane extract would be needed. All of these assumptions were tested through recovery experiments (Section 2.4.1).

2.2 Method development

2.2.1 Sample pre-treatment

Initially the pre-treatment of needle samples was ignored and the direct analysis of whole needles was performed analogous to the HSGC method outlined by Plümacher & Renner (1993). Interestingly it had been noted by Frank et al. (1990) that unavoidable inaccuracies were experienced during standard addition calibrations due to the variability between needles, especially when whole needles were used. The improved extraction of TCAA into water prior to methylation (Norokorpi & Frank 1995) involved the homogenisation of needles by grinding with liquid nitrogen followed by sonication in water. The sonication was not used for MoNKS distillation due to the possibility of TCAA decarboxylation, but the grinding procedure was
implemented for the analysis of needles by the MoNKS method. The high affinity of TCAA for water meant that once the needle matrix and the internal cells had been broken up by grinding the determination of TCAA concentrations was more accurate and reproducible.

The presence of CHCl₃ in the water and adsorbed on the needles not only made its distinction from CHCl₃ produced from the decarboxylation of TCAA difficult, but could have threatened to swamp the contribution from the sample TCAA. The choice of a large mass of needles was used to improve the signal to background ratio, but the removal of background CHCl₃ was still essential for successful analysis. A batch degassing procedure was performed to facilitate this. Seven samples and a water blank were degassed simultaneously using identical flows of OFN. The optimisation of the degassing time was performed as part of method development steps (Section 2.4.3). This meant that a specific water blank determination was associated with 7 sample preparations and was used to correct the results for the TCAA present in the water that was used to prepare the samples. (*N.B. This was not required for aqueous samples i.e. rain or cloud, where no water was added*). If aqueous environmental matrices such as rainwater and cloudwater were analysed by this methodology the only pre-treatment required was degassing with OFN to remove background CHCl₃.

### 2.2.2 Extraction parameters

#### 2.2.2.1 Extraction temperature and time

The aqueous solutions were boiled at 100°C to set up a reflux effect, which gave good cycling of the solutions in the apparatus. This temperature was also used to promote the decarboxylation of any TCAA present. The initial extraction time used was 2 hours, but this was reduced to 1 hour after conversion and recovery experiments proved it optimum (Section 2.4.1.1).

#### 2.2.2.2 Solvent volumes

The addition of a small volume of water to the MoNKS distillation apparatus was important as this allowed the hexane to be added above it, which stopped any hexane
becoming trapped in the tap area. If this happened it would stop that portion of hexane from taking part in the extraction and would reduce the effective volume of hexane used. The initial procedure was to add an unspecified volume of water to the apparatus, but it was realised that the contribution of TCAA from the water could not be ignored. The volume of water was minimised and optimised with respect to standard recovery and conversion experiments (Section 2.4.1.2).

The volume of hexane added was the most critical parameter in this methodology. The volume was added accurately using high grade pipettes. After the addition of hexane the apparatus was assembled and cooled quickly to avoid any loss of hexane by evaporation. The volume of hexane used influenced the concentration of CHCl₃ measured and thus the detection limits. Initially 10 ml hexane was used, but this was reduced to 5 ml to give improved sensitivity. The distillation of aqueous samples were performed using 2 ml hexane volume. The trapping efficiency of these various volumes were tested via recovery experiments (Section 2.4.1.3).

2.2.2.3 Condenser size
To minimise the loss of CHCl₃ from the apparatus during extraction an additional Liebig condenser was attached. Two sizes of condensers were used; long (50 cm) or short (35 cm). The type of condenser used was recorded for each extraction, but no noticeable difference was found when using each condenser size.

2.2.2.4 Nielson-Kryger apparatus efficiency
Each MoNKS distillation apparatus was hand-made by a glass-blower and as such were non-identical. This may have caused slight differences in optimum extraction time due to the trapping characteristics of each condenser. The optimum extraction time was established to be of sufficient length to give 100% conversion and recovery by each MoNKS condenser.
2.2.2.5 Addition of acid

There is the possibility that species other than TCAA might produce CHCl₃ on heating. One such species is chloral hydrate, which may undergo decarbonylation to CHCl₃. It was reported by Køppen et al. (1988) that chloral has a low thermal stability and may be decarbonylated in the blank determination by HSGC. It was also suggested that the decarbonylation of chloral hydrate could be inhibited in the presence of strong acid. It was paradoxically suggested that the decarboxylation of TCAA to CHCl₃ was more reproducible in its acid form (Christensen et al. 1988), but also that decarboxylation only occurred when in its acetate form (Hoekstra et al. 1999b). This conflicting evidence was tested by comparison of the recovery experiments at neutral pH and with 5ml of HNO₃ added (pH<1).

2.2.2.6 Storage of extracts

It was important that once the sample extracts had been prepared that there was no loss of analyte and no contamination by external CHCl₃ during storage. Extracts were initially transferred to 15 ml glass vials with screw caps, which were sealed with nescofilm and stored at 4°C. The procedure used for storage of needle extracts was to fill duplicate 2 ml vials, crimp using PTFE seal caps and to store at 4°C. The vials contained no headspace, which stopped loss of CHC1₃ on injection. Experiments were performed to determine the optimum storage method and sample lifetime (Section 2.4.2).

2.2.3 Gas Chromatographic analysis

The GC method used to analyse hexane for CHCl₃ was a modification of an existing Zeneca analytical method. The use of a column with a ‘624’ phase was consistent with EPA method No. 624 for the analysis of volatile organics. The GC parameters for method 1 & 2, shown in Table 2.1, vary slightly due to the small differences in the dimensions of the column used and their retention times. Both GC method parameters provide a suitable peak shape and resolution from any other peaks present in the chromatogram (Figure 2.2).
<table>
<thead>
<tr>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column type</td>
<td>DB 624</td>
</tr>
<tr>
<td>Dimensions</td>
<td>30m x 0.32 mm x 1.8 μm</td>
</tr>
<tr>
<td>Oven Temp. (1)</td>
<td>50°C</td>
</tr>
<tr>
<td>Time (1)</td>
<td>6 min.</td>
</tr>
<tr>
<td>Rate</td>
<td>40°C min⁻¹</td>
</tr>
<tr>
<td>Oven temp. (2)</td>
<td>150°C</td>
</tr>
<tr>
<td>Time (2)</td>
<td>3 min.</td>
</tr>
<tr>
<td>Injection temp.</td>
<td>200°C</td>
</tr>
<tr>
<td>Split flow</td>
<td>30 ml min⁻¹</td>
</tr>
<tr>
<td>Carrier pressure</td>
<td>12.6 psi</td>
</tr>
<tr>
<td>Detector temp.</td>
<td>375°C</td>
</tr>
<tr>
<td>Make-up gas</td>
<td>N₂</td>
</tr>
<tr>
<td>Attenuation</td>
<td>1</td>
</tr>
<tr>
<td>Injection volume</td>
<td>2 μl</td>
</tr>
<tr>
<td>RT₇₇₇₇</td>
<td>5.08 min.</td>
</tr>
</tbody>
</table>

Table 2.1: GC parameters for analysis of CHCl₃ in hexane

Figure 2.2: Typical Gas Chromatogram for the analysis of CHCl₃ in hexane

The MoNKS distillation method produces extracts with very few components that elute in the isothermal temperature range, but with several unknown late-eluters. An extended temperature programme was used to remove all components from the analytical column between GC cycles. To identify the components extracted by the steam distillation a study of the late eluting components using GC-MS analysis was performed. The components identified are shown in Table 2.2. Several natural products have been identified by GC-MS which probably originate from the needles. It should be noted that no peaks eluted after 23.95 minutes.
<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Component ID</th>
<th>% Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.23</td>
<td>α - Pinene</td>
<td>96</td>
</tr>
<tr>
<td>10.08</td>
<td>β - Pinene</td>
<td>96</td>
</tr>
<tr>
<td>10.38</td>
<td>β - Myrcene</td>
<td>94</td>
</tr>
<tr>
<td>10.60</td>
<td>α - Phellandrene</td>
<td>94</td>
</tr>
<tr>
<td>11.06</td>
<td>β - Phellandrene</td>
<td>90</td>
</tr>
<tr>
<td>12.07</td>
<td>(+) -2- Carene</td>
<td>96</td>
</tr>
<tr>
<td>14.68</td>
<td>2-cyclohexen-1-one</td>
<td>96</td>
</tr>
<tr>
<td>23.95</td>
<td>1-napthalenepropanol</td>
<td>91</td>
</tr>
</tbody>
</table>

Table 2.2; Late eluting components in needle extracts, identified by GC-MS

2.2.4 Efficiency experiments

To test the effectiveness of the extraction parameters described in Section 2.2.2, experiments were performed to investigate changes in conversion and recovery of TCAA with changing extraction parameters. Solutions of TCAA in water were prepared in the range 100-300 ng l⁻¹ and aliquots (100 ml) of these were put through the extraction procedure using the desired parameters. The conversion and recovery of the TCAA was assessed by calibration with external CHCl₃ standards to compare the theoretical with the measured concentration. By investigation of each set of parameters the optimum method was developed.

2.3 Experimental

2.3.1 Sampling procedure

The sampling of needles from Glentress Forest has been regularly performed between 1997 and 2000 using the procedure outlined in Section 4.2. The needle material was stored frozen and was available for analysis by MoNKS distillation. The variations in TCAA concentrations of needles were investigated as outlined in Section 4.3. and were related to spatial and temporal differences at Glentress Forest. For a more complete assessment of the sampling regime see Section 4.3.
2.3.2 MoNKS distillation and GC analysis

The following experimental procedure was used for the preparation of needle samples for the MoNKS distillation and analysis by GC, which was established after method optimisation.

A large mass of whole needles was weighed so that several duplicate extractions could be performed, each of which required a 5 g sample. The sample was transferred to a mortar, liquid nitrogen was added and the needles were ground to a powder using a pestle. The sample was allowed to equilibrate at room temperature and 5 g was accurately weighed into a RBF. To the flask 50 ml deionised water was added. Seven sample solutions and a blank solution containing 50 ml deionised water were simultaneously degassed for 1 hour using OFN. The sample was then connected to the apparatus using a quickfit clip. To the MoNKS apparatus 25 ml of degassed deionised water and 5 ml hexane were added using ‘A’ grade glass pipettes. A Liebig condenser was attached to the top of the apparatus, and the system as a whole was cooled via the cooling jacket. The RBF was heated using a heating mantle for 1 hour after which time the apparatus was allowed to cool. The water phase was drawn off and the hexane extract collected through the tap. Two vials (2 ml) were filled with extract, capped with PTFE seals and stored at 4°C until analysis. Automatic GC-ECD analysis of the extracts was performed using a Perkin-Elmer Autosystem with automatic injection using the parameters outlined in Table 3.4. The CHCl₃ peak areas were determined using Hewlett-Packard Workstation software.

2.3.3 Calibration and calculation of results

The CHCl₃ concentration of the extracts were determined by GC-ECD. External CHCl₃ standards in hexane were prepared in the range of 0 to 30 ppb CHCl₃ and analysed by GC-ECD for calibration. At the start of the analysis a set of 2 independently prepared CHCl₃ standards were analysed for standard recovery. During the analysis sequence a CHCl₃ standard vial and hexane blank were injected after every 10 samples to determine the response factor (RF) of the ECD to CHCl₃.
A hexane blank was stored and analysed for each day or for each batch of extractions. This meant that the CHCl₃ background in hexane specific to certain sample extracts could be corrected for to determine the increase in CHCl₃ during the MoNKS distillation. For calculation of results the peak area of the hexane blank was subtracted from the relevant sample peak areas to give the hexane-corrected peak area. Each set of 7 samples were associated with a specific water extract (Section 2.2.1) and so the hexane-corrected peak area of the water extract was subtracted from the hexane-corrected sample areas to give the water-corrected areas. If these areas were divided by the RF, the CHCl₃ concentration in the extract was calculated. This concentration was then related to the TCAA present in the original sample.

Using the Equation 2.2 the TCAA present in the needle sample (in ng g⁻¹) or the aqueous sample (ng l⁻¹) was calculated.

\[
\text{TCAA (ng g}^{-1}\text{)} = \frac{\text{Conc. CHCl}_3 \text{ (ppb)} \times 1.369 \times 0.005 \times 1000}{\text{mass sample (g)}}
\]

(Equation 2.2)

which can be simplified to;

\[
\text{TCAA (ng g}^{-1}\text{)} = \frac{(\text{Conc. CHCl}_3 \text{ (ppb)} \times 6.845)}{\text{mass sample (g)}}
\]

(Equation 2.3)

This equation holds for extractions using 5 ml of hexane, but if a 2 ml volume is used then the Equation 2.2 has 0.002 in the numerator instead. Similarly if an aqueous sample is used division by sample volume in litres and not sample mass should be performed.
2.4 Results

2.4.1 Recovery and conversion experiments

2.4.1.1 Effect of extraction time on efficiency

The most important factor to be optimised in the MoNKS distillation was the extraction time. The recovery is influenced by a number of factors which are time-dependent such as the time required for the total decarboxylation of TCAA and for the solvent to completely extract CHCl₃ from the steam distillate. By the choice of an appropriate extraction time many of the factors can be negated and the overall efficiency is then determined by recovery efficiency of the trapping solvent. It was suggested by Frank et al. (1990) that by using HSGC analysis total decarboxylation was possible at 100°C for 2 hours, so it was assumed that these conditions would be appropriate in the MoNKS method. It was desirable to reduce the extraction time further to enable higher sample throughput, so reduced extraction times were also tested.

A solution of 50 ml deionised water was spiked with 500 μl of 197 ppb TCAA solution and was extracted into 5 ml hexane. This was performed in triplicate for 1 and 2 hour extraction times. The actual CHCl₃ concentration of the extract was compared with the theoretical maximum of 14.4 ppb. The results are shown in Table 2.3.

<table>
<thead>
<tr>
<th>TCAA standard</th>
<th>1 hour extraction</th>
<th>2 hour extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>197 ppb</td>
<td>13.5 ± 2.2 ppb</td>
<td>12.1 ± 0.9 ppb</td>
</tr>
<tr>
<td></td>
<td>(Recovery: 94 ± 15%)</td>
<td>(Recovery: 85 ± 6%)</td>
</tr>
</tbody>
</table>

Table 2.3: Effect of extraction time on recovery of TCAA
(Results are mean ± standard deviation of triplicate analysis)

The 1 hour extraction time appears to give the optimum conversion and recovery of TCAA, which is reduced if continued for 2 hours. The reason for this could be that the extended extraction might lose some CHCl₃ from the apparatus thus giving the appearance of incomplete conversion. The 1 hour extraction clearly demonstrates
that complete conversion is possible in a shorter time. A repeat of the 2 hour extraction gave a similar recovery \((85 \pm 8\%; n=3)\) suggesting that the same losses were occurring. This optimum extraction time only applies to these extraction parameters, so if a smaller hexane volume was used another recovery experiment must be performed to determine its optimum time. One such experiment using 2 ml hexane was required. The recovery for 1 hour extractions were much less than 100%, but for 2 hour extractions of 50 ml of 310 ppt TCAA solution a recovery of \(111 \pm 9\%\) was achieved. This showed that 2 hour extractions gave optimum recovery using certain extraction conditions. Later experiments (Section 2.4.1.3) showed that 1 hour extractions are sufficient when using both 2 ml and 5 ml hexane, but absolute care must be taken to ensure perfect seals in the glass connections to prevent any outward leaks or ingress of contaminants. The established method for needle extractions proved that the optimum extraction time was 1 hour, which gave maximum conversion and recovery of TCAA with the shortest analysis time.

### 2.4.1.2 Effect of water volume on recovery

It was discussed in Section 2.2.2.2 that due to possible trapping of hexane in the tap area a volume of water had to be added to the apparatus prior to the addition of the volume of hexane. It was also suggested that because the bottom of the condenser could become hot the hexane may be lost due to evaporation. If water was added first, then the hexane would not come into contact with any hot surfaces. This water was degassed so as not to introduce CHCl₃ into the system, but it was desirable to minimise the blanks in the extraction to improve the detection limits and sensitivity. For this reason experiments were performed using 1, 10 and 20 ml of deionised water in the apparatus to test their effect on recovery.

Using the water volumes stated above 50 ml of 310 ppt TCAA solutions were extracted into 5 ml hexane using an extraction time of 1 hour. The recovery results are shown in Table 2.4.
The volume of water does not significantly alter the recovery of TCAA in MoNKS distillation procedure. With all volumes the recovery is close to 100% and because the same volume is added to the extraction of the water blank any CHCl₃ or TCAA present can be corrected for. No test has been performed using zero added water, but as previously discussed for practical reasons this is not a worthwhile exercise. This experiment also demonstrates the correct selection of 1 hour as the extraction time as 24 extractions demonstrate good accuracy and precision.

<table>
<thead>
<tr>
<th>Volume of water (ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93 ± 15%</td>
</tr>
<tr>
<td></td>
<td>(n=9)</td>
</tr>
<tr>
<td>10</td>
<td>110 ± 9%</td>
</tr>
<tr>
<td></td>
<td>(n=9)</td>
</tr>
<tr>
<td>20</td>
<td>105 ± 11%</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
</tr>
</tbody>
</table>

Table 2.4; Effect of the water volume added to condenser on recovery

(Err ranges are 95% confidence intervals. n = no. replicates)

2.4.1.3 Effect of hexane volume on recovery

The purpose of the hexane phase is to partition any CHCl₃ passing through it from aqueous or gas phase. The dimensions of the condensers determine the height of the hexane layer and so affect the contact time and contact area of the hexane with any CHCl₃. However, the choice of hexane volume affects both the extraction efficiency and the concentration factor achieved post-extraction. Differing volumes alter the time taken to achieve 100% recovery and so each must be tested using the other set extraction parameters. As discussed in Section 2.4.1.1, 5 ml hexane was able to give 100% recovery using 1 hour extraction time whereas 2 ml of hexane required 2 hours. The results from more tests on the effect of hexane volume are reported here.

The previous results in Section 2.4.1 have proved that using 5 ml of hexane with a 1 hour extraction time gave optimum recovery, but this volume of hexane may not always be appropriate. If lower concentrations must be determined and the sample mass/volume is limited i.e. rainwater, the main way of lowering the detection limit is
to use a smaller hexane volume. The results in Table 2.5 show recoveries from extractions using 2 ml hexane.

<table>
<thead>
<tr>
<th>TCAA standard</th>
<th>Recovery (%)</th>
<th>No. replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>198 ppb (250 μl)</td>
<td>99 ± 14%</td>
<td>3</td>
</tr>
<tr>
<td>444 ppt (50 ml)</td>
<td>98 ± 5%</td>
<td>4</td>
</tr>
<tr>
<td>606 ppt (50 ml)</td>
<td>107 ± 25%</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2.5; Recovery of TCAA using a 2 ml hexane extraction volume

(Error ranges are standard deviations of n. replicates. The concentration of the TCAA stock solution is shown, with the volume used in parentheses)

These results show that complete conversion and recovery of TCAA into 2 ml hexane using a 1 hour extraction time is also possible. It should be noted that when using only 2 ml hexane the experimental errors are very critical. Any error in the volume of hexane used, or any loss of hexane during the extraction is magnified more so than if 5 ml hexane were used. Another similar experiment determined only 50% recovery so robust laboratory procedures must be used. As shown in Table 2.5 solutions in the 0.5 ppb TCAA range can be analysed yielding extracts with CHCl₃ concentrations in the 10 ppb range, which can be routinely analysed by GC with high precision and accuracy. If this procedure is used routinely it is advisable to perform periodic TCAA standard extractions to check for correct extraction procedure and 100% recovery. If unlimited sample is available then 5 ml hexane volume is more reliable.

2.4.1.4 Effect of acid on recovery

The only direct experiment comparing the recovery achieved with and without the addition of acid was performed after the extraction parameters were established. TCAA solution (50 ml) was extracted in triplicate into 5 ml hexane. An identical set of 3 extractions had 2.5 ml HNO₃ (11 M) added. The results are shown in Table 2.6 where 100% conversion theoretically gave 14.4 ppb CHCl₃.
<table>
<thead>
<tr>
<th>TCAA standard</th>
<th>No acid extraction</th>
<th>Acid extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>197 ppb</td>
<td>13.5 ± 2.2 ppb</td>
<td>10.4 ± 1.1 ppb</td>
</tr>
<tr>
<td></td>
<td>(Recovery: 94 ± 15%)</td>
<td>(Recovery: 72 ± 8%)</td>
</tr>
</tbody>
</table>

Table 2.6; Effect of acidity on conversion of TCAA

*(Error ranges are standard deviations on triplicate analyses)*

This experiment suggests that the decarboxylation of TCAA is inhibited when in its acid form. It is significant as the extractions and analysis were performed on the same day so there is no chance of the difference being caused by changing response factors or blank values. This is a only a single experiment and does not conclusively prove that acid causes reduced conversion and recovery of TCAA.

2.4.1.5 Effect of MoNKS distillation apparatus on recovery

The individual condensers have slightly different dimensions, but their efficiency has indirectly been tested throughout the development stages. The results shown in Table 2.4 have tested the recoveries of 9 different MoNKS apparatus in two separate experiments. The results for 1 ml and 10 ml of water used different apparatus and condensers for each extraction. The recoveries in both experiments are accurate and precise, which suggests that the established extraction parameters are sufficient for 100% conversion and recovery of CHCl₃ in any MoNKS condenser.

2.4.2 CHCl₃ Standard storage experiments

The importance of the stability and integrity of the CHCl₃ standards and extracts has already been discussed. The effect of the mode of storage on concentration was investigated using a stock standard solution of 5 ppb CHCl₃ in hexane. The standard was stored in either a 2 ml vial with rubber (R) or PTFE (T) inserts in the cap or in a 15 ml glass screw-cap vial (V). The storage amount was either full with no headspace (no prefix) or half full (H). The vials were stored either at 4°C in a refrigerator (no prefix) or at room temperature (RT). The samples were analysed on two occasions either initially (time 0) or after 13 days storage (time 2). The results are shown in Table 2.7 & 2.8.
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Area 1</th>
<th>Area 2</th>
<th>Average area</th>
<th>Conc. CHCl₃ (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane blank</td>
<td>65606</td>
<td>67397</td>
<td>66502</td>
<td>-</td>
</tr>
<tr>
<td>Time0 1T</td>
<td>229776</td>
<td>230406</td>
<td>230091</td>
<td>5.42</td>
</tr>
<tr>
<td>Time0 2T</td>
<td>217835</td>
<td>228867</td>
<td>223351</td>
<td>5.20</td>
</tr>
<tr>
<td>Time0 1R</td>
<td>228260</td>
<td>230103</td>
<td>229182</td>
<td>5.39</td>
</tr>
<tr>
<td>Time0 2R</td>
<td>235457</td>
<td>238583</td>
<td>237020</td>
<td>5.66</td>
</tr>
<tr>
<td>Mean ± std dev</td>
<td>229911 ± 6046</td>
<td>5.42 ± 0.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Response Factor: 30165

Table 2.7: Peak area at time 0 for CHCl₃ storage experiment

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Area 1</th>
<th>Area 2</th>
<th>Average area</th>
<th>Conc. CHCl₃ (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time2 Hex1T</td>
<td>62595</td>
<td>63447</td>
<td>63021</td>
<td>-</td>
</tr>
<tr>
<td>Time2 Hex2T</td>
<td>60077</td>
<td>62428</td>
<td>61253</td>
<td>-</td>
</tr>
<tr>
<td>Time2 1T</td>
<td>213220</td>
<td>212058</td>
<td>212639</td>
<td>5.41</td>
</tr>
<tr>
<td>Time2 2T</td>
<td>210876</td>
<td>211870</td>
<td>211373</td>
<td>5.36</td>
</tr>
<tr>
<td>Time2 1R</td>
<td>202156</td>
<td>191150</td>
<td>196653</td>
<td>4.84</td>
</tr>
<tr>
<td>Time2 1HT</td>
<td>207233</td>
<td>210270</td>
<td>208752</td>
<td>5.27</td>
</tr>
<tr>
<td>Time2 2HT</td>
<td>199824</td>
<td>207862</td>
<td>203843</td>
<td>5.09</td>
</tr>
<tr>
<td>Time2 1HR</td>
<td>208124</td>
<td>215600</td>
<td>211862</td>
<td>5.38</td>
</tr>
<tr>
<td>Time2 2HR</td>
<td>213602</td>
<td>200776</td>
<td>207189</td>
<td>5.21</td>
</tr>
<tr>
<td>Time2 1RRT</td>
<td>214517</td>
<td>212883</td>
<td>213700</td>
<td>5.45</td>
</tr>
<tr>
<td>Time2 2RRT</td>
<td>208229</td>
<td>211098</td>
<td>209664</td>
<td>5.30</td>
</tr>
<tr>
<td>Time2 1RTR</td>
<td>201639</td>
<td>204434</td>
<td>203037</td>
<td>5.07</td>
</tr>
<tr>
<td>Time2 2RTR</td>
<td>202223</td>
<td>198906</td>
<td>200565</td>
<td>4.98</td>
</tr>
<tr>
<td>Time2 1HRTT</td>
<td>202058</td>
<td>200878</td>
<td>201468</td>
<td>5.01</td>
</tr>
<tr>
<td>Time2 2HRTT</td>
<td>202998</td>
<td>201896</td>
<td>202447</td>
<td>5.04</td>
</tr>
<tr>
<td>Time2 1HRTR</td>
<td>213590</td>
<td>209569</td>
<td>211580</td>
<td>5.37</td>
</tr>
<tr>
<td>Time2 2HRTR</td>
<td>207236</td>
<td>(161058)</td>
<td>207236</td>
<td>5.22</td>
</tr>
<tr>
<td>Time2 1V</td>
<td>225532</td>
<td>220826</td>
<td>223179</td>
<td>5.79</td>
</tr>
<tr>
<td>Time2 1VRT</td>
<td>214499</td>
<td>215773</td>
<td>215136</td>
<td>5.50</td>
</tr>
</tbody>
</table>

Response Factor: 27820

Table 2.8: Peak areas at time 2 for CHCl₃ storage experiment
The time 2 results were analysed statistically by taking the average peak areas as the variable, and performing ANOVA and Tukey’s studentised range test. This statistical test was used to determine if there were any significance differences between storage according to their fill level, temperature and cap type.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>ANOVA SS</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill</td>
<td>1</td>
<td>$1.8 \times 10^{-7}$</td>
<td>$1.8 \times 10^{-7}$</td>
<td>0.00</td>
<td>0.9796</td>
</tr>
<tr>
<td>Temp.</td>
<td>1</td>
<td>$2.46 \times 10^{-4}$</td>
<td>$2.46 \times 10^{-4}$</td>
<td>0.90</td>
<td>0.3520</td>
</tr>
<tr>
<td>Cap</td>
<td>1</td>
<td>$9.34 \times 10^{-4}$</td>
<td>$2.46 \times 10^{-4}$</td>
<td>3.43</td>
<td>0.775</td>
</tr>
<tr>
<td>Fill * Temp.</td>
<td>1</td>
<td>$2.94 \times 10^{-4}$</td>
<td>$2.94 \times 10^{-4}$</td>
<td>1.08</td>
<td>0.3099</td>
</tr>
<tr>
<td>Fill * Cap</td>
<td>1</td>
<td>0.0103</td>
<td>0.0103</td>
<td>37.72</td>
<td>0.0001</td>
</tr>
<tr>
<td>Temp. * Cap</td>
<td>1</td>
<td>$8.78 \times 10^{-4}$</td>
<td>$8.78 \times 10^{-4}$</td>
<td>3.22</td>
<td>0.0863</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>0.00599</td>
<td>$2.72 \times 10^{-4}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.9: ANOVA results for stored CHCl$_3$ standard vials (Time 2)

The statistical analysis shows that no significant difference exists between the storage of vials at the different conditions of fill, temperature or cap type. However, the ANOVA has shown a significant ($P=0.0001$) interaction between cap type and fill level which is not fully understood. The peak area of vial Time2 2R was overloaded (not shown in Table 2.8), which suggested that there was contamination of the vial by CHCl$_3$. It is impossible to say whether this occurred at the start of the experiment or during storage. There is a weakly significant ($P<0.1$) difference between the cap types with rubber caps giving slightly lower concentrations than PTFE caps.

The glass vials (V) displayed the highest concentrations at time 2. It is unsure why this should be the case as vials with a large headspace would be expected to lose CHCl$_3$ on opening, which has not proved to be the case. These results have not been analysed statistically due to the small number of vials kept by this storage type.

When considering the concentrations of CHCl$_3$ in the vials at time 2 there are generally lower concentrations than time 0. This absolute change may be influenced by differences in the response factor which may be true or caused by errors in the
preparation of the calibration standards. The mean CHCl₃ concentration at time 0 was 5.42 ± 0.19 ppb compared with 5.20 ± 0.18 ppb at time 2 (only 2 ml vials averaged). This experiment has investigated statistical differences between the concentrations in the stored vials rather than the changes from time 0 to time 2. The results of this experiment suggests that any of the tested storage protocols could be used, and also validates the established procedure of storing the extracts in filled, PTFE-capped vials in a refrigerator. The results suggest that no significant change should be seen over a two week period.

### 2.4.3 Degassing efficiency experiments

For the extraction method to give accurate results for TCAA analysis of real samples it must be able to remove any CHCl₃ present on the needle samples. This can be done by degassing the solution containing the sample with OFN for a set time period. This set of experiments was designed to test whether the degassing protocols were capable of removing certain concentrations of CHCl₃ from spiked solutions of TCAA and water.

The initial experiment was performed to investigate the ability of degassing to remove a CHCl₃ spike from water and from a TCAA standard solution. Preparation of the water spikes was by the addition of 3 µl of 12 ppm CHCl₃ in methanol solution to 100 ml deionised water. Extraction was performed into 5 ml hexane resulting in a theoretical final extract concentration of 7.2 ppb CHCl₃. Spiked standards were produced from 100 ml of 300 ppt TCAA solution with the same CHCl₃ spike as above. This theoretically produced a final extract concentration of 11.6 ppb CHCl₃, from contributions of 4.4 ppb from TCAA standard and 7.2 ppb from the CHCl₃ spike. The aim of the experiment was to attempt to remove the spikes after 1 or 2 hours degassing. The results are shown in Table 2.10.
The degassing of the spiked water samples clearly demonstrated the removal of the spiked CHCl₃ within 1 hour. It was also obvious that the theoretical 7.2 ppb spike was actually smaller than theory, possibly due to loss during spiking or a low CHCl₃ stock standard. The CHCl₃ spike in the spiked standard confirmed this assumption as the difference between the initial sample and the two degassed samples was 2.69 and 2.79 ppb respectively. This also demonstrated that all CHCl₃ was removed in 1 hour as a further hour led to no additional decrease in concentration. There was agreement in the magnitude of the TCAA standard, which is similar between the spiked and unspiked TCAA after 1 and 2 hours of degassing. However, the non-degassed standard exhibits an average concentration of 4.61 ppb, which is in excellent agreement with the theoretical value of 4.38 ppb. There is no explanation for the difference in the determined TCAA concentration, but the effectiveness of degassing was proved. Further experiments were designed to repeat these findings.

Another spiking experiment was performed, giving theoretical extract concentrations of 16.5 ppb CHCl₃ for the spiked standard and 6.0 ppb for spiked water. The results are shown in Table 2.11.

<table>
<thead>
<tr>
<th>Degassing time (hours)</th>
<th>CHCl₃ spiked in water (ppb)</th>
<th>CHCl₃ spiked in TCAA (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.4 ± 0.4</td>
<td>14.7 ± 3.4</td>
</tr>
<tr>
<td>1</td>
<td>nd</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>nd</td>
<td>6.8 ± 1.1</td>
</tr>
</tbody>
</table>

Table 2.11; Effect of degassing on removal of CHCl₃ spike
(Results are mean ± standard deviation on triplicate analysis. nd = not detected)
The effectiveness of the degassing procedure is shown in Table 2.11, however the recovery of the TCAA standard was poor. The spiked water produced undetectable levels of CHCl₃ after degassing for only 1 hour. The concentration of the spiked standard was reduced to a constant level after only 1 hour, which suggests that all CHCl₃ present was removed. The aim of the experiment was achieved as it showed that by degassing for 1 hour with OFN any CHCl₃ either introduced into the solution, adsorbed to the needles or in the deionised water could be volatilised and removed. It also illustrated the problems of calibration and the need to maintain high recovery in order to produce accurate results. A good example of an accurate spiking experiment is shown in Table 2.12.

<table>
<thead>
<tr>
<th>Degassing time (hours)</th>
<th>Conc. CHCl₃ (ppb)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29.4 ± 1.2</td>
<td>92 ± 9%</td>
</tr>
<tr>
<td>2</td>
<td>23.6 ± 3.0</td>
<td>91 ± 28%</td>
</tr>
</tbody>
</table>

Table 2.12; Effect of degassing on removal of CHCl₃ spike

(Results are mean ± standard deviation on triplicate analysis)

The theoretical CHCl₃ concentrations were 31.9 ppb and 25.9 ppb for spiked and unspiked standard respectively. The recovery at both degassing times was excellent (>90%) and demonstrated both degassing efficiency and optimum conversion and recovery of TCAA. This illustrates the capabilities of this method with the established protocols.

2.4.4 Measurements of TCAA in needles

2.4.4.1 Tree-to-tree variation

Needle TCAA concentrations may vary from tree-to-tree over very small distances within a forest. Many factors may be responsible for this variation including tree size, position within a stand, prevailing wind direction etc.. For this reason it was important to understand the variation of TCAA within an individual sampling site and also the method reproducibility for the analysis of foliage samples. The aim of this
The experiment was to determine whether needle samples could be pooled to give a representative sample at a site or if analysis of several individual trees was required.

Needle samples were taken at the beginning of the needle growing season soon after bud burst and the subsequent elongation of the needles. It was expected that this would give a truer representation of the natural tree-to-tree variation before the needles were affected by atmospheric deposition. Freshly opened current year needle buds (1998 growing season) were taken from 7 Sitka spruce trees, 5 at Dunslair Heights and 2 at Venlaw. This also allowed a comparison between the needle concentrations at sites of different altitudes, which experience different amounts of wet deposition. Table 2.13 gives a description of the position of the individual trees within the forest.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sampling site</th>
<th>Tree position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree 1</td>
<td>DH</td>
<td>Stand edge at fire break</td>
</tr>
<tr>
<td>Tree 2</td>
<td>DH</td>
<td>Stand edge at fire break</td>
</tr>
<tr>
<td>Tree 3</td>
<td>DH</td>
<td>In stand by clearing</td>
</tr>
<tr>
<td>Tree 4</td>
<td>DH</td>
<td>In stand by sampler</td>
</tr>
<tr>
<td>Tree 5</td>
<td>DH</td>
<td>Stand edge at path</td>
</tr>
<tr>
<td>Tree 6</td>
<td>Venlaw</td>
<td>Above road, 3 rows into stand</td>
</tr>
<tr>
<td>Tree 7</td>
<td>Venlaw</td>
<td>Below road, 3 rows into stand</td>
</tr>
</tbody>
</table>

Table 2.13: Position of sampled trees within Glentress Forest

Several needle buds were sampled from the same tree and pooled before analysis by the MoNKS distillation. Replicate extractions were performed and the hexane extracts were analysed by GC-ECD as outlined in Section 2.3.2. The concentration of TCAA in the needles relative to fresh weight was calculated and is shown in Figure 2.3.
In order to determine whether the differences in TCAA between trees were significant or caused by within sample variations a single factor ANOVA was performed, the results of which are shown in Table 2.14.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between trees</td>
<td>5411.2</td>
<td>6</td>
<td>901.9</td>
<td>4.72</td>
<td>0.0014</td>
<td>2.38</td>
</tr>
<tr>
<td>Within trees</td>
<td>6494.7</td>
<td>34</td>
<td>191.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11905.9</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.14; Single factor ANOVA results for tree-to-tree TCAA variation

The statistical test shows evidence of significantly greater variation between tree samples than between replicate analyses proving that there is a significant difference between the TCAA concentrations in the individual trees. These results highlight two important points. Firstly, the analytical procedure is precise enough to allow the
observation of natural variations in TCAA between samples due to the excellent analytical reproducibility. Secondly the trees, even at bud burst, display variable TCAA concentrations in their needles. This may be explained by meteorological conditions due to the trees’ positions within a stand or forest, which influences the exposure to an input of atmospheric pollutants. Another influence is the size of the tree, with larger trees transpiring larger volumes of water and thus possibly taking up more TCAA from the soil via its transpiration stream. As is seen in Chapter 5 the spatial variability of TCAA in soil is large due to the heterogeneity of soil, which influences the TCAA available for uptake by each tree. The implications of these findings is that it would be incorrect to sample a single tree at each site and assume it is representative of that sampling site. For this reason the sampling protocol has involved the sampling of 5 branches from different trees at different areas of each sampling site. A sample of each needle year class was taken from each branch and pooled, which when analysed gave a representative TCAA concentration for that site.

The results shown in Figure 2.3 were used to calculate the mean of the means at Dunsclair Heights (trees 1-5) and at Venlaw (trees 6 & 7) and it can be seen that the TCAA concentrations at each site are very similar (Table 2.15). Despite the fact that wet input of TCAA to Dunsclair Heights is 50% greater than at Venlaw (Table 6.12) the concentrations in the needles are very similar, which suggests that there is either another source of TCAA, such as dry deposition or soil production, or that some internal destruction/ metabolic processes maintain the constant needle TCAA concentrations. From this limited data it is not possible to differentiate which of these is more likely, but a more detailed study of TCAA cycling in forests is reported in Chapter 4.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>TCAA (ng/ g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunsclair Heights</td>
<td>61.5 ± 13.3</td>
</tr>
<tr>
<td>Venlaw</td>
<td>64.3 ± 9.0</td>
</tr>
</tbody>
</table>

Table 2.15; Mean needle TCAA concentrations at Glentress Forest

(Error range is standard deviation of mean tree TCAA concentrations)
It should also be noted that the concentrations in the needles analysed during the bud burst period are much higher than measured during the later months. This dilution effect is well known and was studied in detail in Section 4.5.1 during the 1999 growing season. The analyte of interest is diluted due to needle elongation during the growing phase of the tree (Linder & Flower-Ellis 1989).

2.4.4.2 Temporal patterns of TCAA at Glentress Forest

The results shown in Figure 2.4 & 2.5 are the patterns of TCAA concentrations in needles at the two sampling sites in Glentress Forest during the 1998 growing season. The results are reported in ng TCAA per g fresh weight of sample and the error bars are 95% confidence intervals. Tentative trends may be drawn from the data though the set is limited and any hypotheses should be tested over a longer time period.

The patterns are quite different in the data from the two different sites. At Dunslair Heights TCAA concentrations appear to increase with increasing needle year class, whereas at Venlaw no such pattern is observed, with similar concentrations in the C to C+3 year classes of needles. Previous work by Frank et al. (1990) suggested that older needles always exhibited higher TCAA concentrations than younger ones. There is tentative evidence at Dunslair Heights to support this claim, but the same
theory does not hold at Venlaw. It seems that there are either different input processes or magnitude of TCAA sources at the two sites or otherwise different removal/degradation processes, which produce these distinct patterns in the data. This was investigated further in Chapter 4.

Figure 2.5; TCAA concentrations in needles at Venlaw

(Results are means ± 95% confidence intervals on triplicate analyses)

The TCAA concentrations in year C needles at the two sites are not significantly different, which supports the findings from the bud burst concentrations where there were also very similar concentrations at the two sites (Section 2.4.4.1). If the input of TCAA to trees was solely via wet deposition then a higher needle concentration at Dunslair Heights would be expected, as the input of TCAA by wet deposition is greater at the upper site (Chapter 6). The fact that this has not been observed suggests there may be other sources of TCAA or other processes occurring. Another feature of the pattern is the apparently higher concentrations of TCAA in winter than in summer in year C needles at both sites. A maximum concentration of TCAA in needles and wet deposition during summer has been observed in Germany (Frank et al. 1990), which was explained by a possible increased atmospheric formation of TCAA from its chlorinated solvent precursors due to higher solar intensity. This
pattern is not observed at Glentress Forest in this limited data, but should be monitored over longer time periods with more frequent sampling (see Chapter 4). If the needle TCAA concentrations for a certain year class of needles are monitored over time then variations can be related either to accumulation or degradation/metabolism or both. In Figure 2.6 all the results from the analysis of current year needles from the 1997 growing season are shown, which illustrates that over the whole period the TCAA concentrations have fluctuated. Figure 2.6 also suggests that the maximum concentrations are measured during the winter months and that the summer months produce the minimum TCAA concentrations. One explanation for this phenomenon could be decreased degradation/metabolism rates during winter due to the lower temperatures, with a subsequent build-up of TCAA in needles. This hypothesis must be tested with a larger data set examining the complementary meteorological data.

![Figure 2.6; Temporal TCAA trend in 1997 needles at Dunslair Heights](image)

*(Error bars are 95% confidence intervals on triplicate analyses)*

### 2.4.4.3 The effect of pre-treatment on needle TCAA determination

Previous work (Frank *et al.* 1990) has reported that grinding of needle material prior to analysis produced more reproducible results. An investigation was performed to test whether the homogenisation of needles prior to the MoNKS distillation produced
any significant difference in the TCAA concentrations determined. Figure 2.7 shows a comparison between the results obtained for ground and whole needles.

![Bar chart showing TCAA concentrations for different sample IDs](image)

**Figure 2.7; The effect of homogenisation on determined TCAA concentrations in needles by MoNKS distillation**

*Error bars are 95% confidence intervals on triplicate analyses*

Figure 2.7 suggest that by grinding needles with liquid nitrogen higher levels of TCAA were determined. The main reason for this could be the location of TCAA within a needle. Recent work by Matucha *et al.* (2000a) has shown that isotopically labelled TCAA is located in the internal matrix of a needle, which is not easily removed or leached. It is possible that boiling whole needles in water for an hour is not severe enough conditions to extract TCAA from the needle matrix and so may lead to incomplete TCAA determination. The homogenisation process immersed the needles in liquid nitrogen causing the cells of the needles to be ruptured. The subsequently milling in a pestle and mortar, left the TCAA free to move into the water matrix to be determined by decarboxylation. A benefit of this procedure is to produce more reproducible results due to the more consistent extraction. The results in Figure 2.7 do not show improved reproducibility, but in the majority of samples analysed by MoNKS the RSD is less than 10%, which is very satisfactory. All of the results from
needles extractions in this chapter have used this homogenisation procedure prior to MoNKS distillation to enable accurate determination of TCAA.

2.4.4.4 Needle washing experiments

The exact location of TCAA in Sitka spruce needles is not well understood. It has been suggested that TCAA might enter needles via liquid junctions at the stomata (Burkhardt & Eiden 1994), and conversely the explanation for increased TCAA concentrations in throughfall was leaching of TCAA out of needles. A simple experiment was performed to determine the location of TCAA in needles, and the effect of different pre-treatments. Whole and ground needles were determined as outlined previously, but also needles were washed with water, to remove any surface-bound TCA.A, and hexane to remove the entire cuticle. Frank et al. (1990) has suggested that the chlorinated solvent precursors are taken up by the waxy cuticle and can subsequently be converted to TCAA by solar radiation. The removal of the cuticle by hexane washing for 10 seconds was designed to remove the waxy cuticle to distinguish any TCAA present in it. The results are shown in Table 2.16.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>54.7 ± 1.3</td>
<td>6.9 ± 1.7</td>
</tr>
<tr>
<td>Water washed</td>
<td>33.6 ± 7.8</td>
<td>8.3 ± 4.7</td>
</tr>
<tr>
<td>Hexane washed</td>
<td>27.0 ± 2.4</td>
<td>6.2 ± 2.5</td>
</tr>
<tr>
<td>Ground</td>
<td>30.1 ± 3.2</td>
<td>5.4 ± 2.1</td>
</tr>
</tbody>
</table>

Table 2.16; Needle TCAA concentrations (ng g⁻¹ fwt.) after various pre-treatments

*Error ranges are standard deviations of triplicate analyses*

The results from the pre-treatment experiments are shown in Table 2.16, where experiment 1 used needles from Dunslair Heights and experiment 2 used needles from Venlaw. In the first experiment the results for the analysis of the ‘as-received’ whole needles are higher than any of the subsequent determinations. There are two possible explanations for this: firstly, it appears by comparison of the whole with the water washed results that 20 ng (g fwt.)⁻¹ TCAA is removed by washing with water, which
suggests that TCAA is present as surface-bound TCAA. This contribution is variable depending on recent weather conditions at the site and wetting and drying cycles of TCAA to the surface. It is surprising that the contribution is so large, but illustrates why the needles are routinely rinsed before analysis. The water-washed, hexane-washed and ground needle concentrations were not significantly different. Even though the cuticles of the needles were removed there was an insignificant decrease in TCAA, which provides further evidence that TCAA is stored within the cells of the needles. Experiment 2 did not show significant changes between pre-treatments, which suggests that the contribution of surface-bound TCAA is less in this batch of needles, which probably varies from tree to tree or site to site. This simple washing experiment provides tentative evidence for the presence of TCAA within the interior of needles, which cannot easily be removed by washing.

2.4.5 TCAA analysis of other environmental matrices

The MoNKS distillation can be used for environmental matrices other than needles. Theoretically any sample that can be homogenised and slurried with water can be analysed, but the most applicable matrix is aqueous samples. Preliminary work with soil has shown that there are problems with the foaming and bumping of soil samples in the apparatus, but this could be solved with additional method development. Aqueous samples are ideal for MoNKS distillation because unlimited sample volumes may be extracted. The ability to pre-concentrate TCAA from 1 litre of sample into 5 ml of hexane means that very low detection limits may be achieved. However, the limitation of this method is when only very small sample volumes are available i.e. <20 ml. Rain, river, lake and sea samples are unlikely to be limited to very small volumes, but matrices such as cloudwater and pore-water may be. The performance of the method is demonstrated by the analysis of samples from various environmental matrices, with the determined concentrations shown in Table 2.17.
These measurements illustrate the ability of the method to determine concentrations as low as 0.2 ppb TCAA if sufficient sample is available. The concentrations in Table 2.17 are also in good agreement with those found in other European studies (Table 6.1) and suggest a framework for future work. The replicate measurements of the pond sample have a relative standard deviation of 15%, which is acceptable at such low concentrations but can probably be improved.

### 2.4.6 Comparison of results from MoNKS and HSGC

A comparison between the concentrations of TCAA determined by MoNKS distillation and HSGC was performed to estimate the accuracy of the two methods. Both methods rely on the decarboxylation of TCAA to CHCl₃, and as such, true accuracy would require comparison with another selective technique, such as derivatisation and analysis by mass spectrometry, but this comparison still serves a useful purpose. Both techniques were run side by side for only a short period, which meant that much of the HSGC analysis was performed on the same samples that had been frozen during the interim period. No degradation was thought to have occurred during freezing.

The results in Figure 2.8 show a good comparison between the two analytical methods. There are no statistical differences between the results by MoNKS distillation and HSGC analysis. This implies that the accuracy of the newly developed MoNKS method is acceptable compared to the established HSGC method which also used decarboxylation. A more comprehensive measure of accuracy would be by comparison with a mass spectrometry technique that positively identified each

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample type</th>
<th>TCAA (ng l⁻¹)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deepsyke</td>
<td>Pond</td>
<td>736 ± 112</td>
<td>4</td>
</tr>
<tr>
<td>Dunslair Heights</td>
<td>Cloud</td>
<td>633; 574</td>
<td>2</td>
</tr>
<tr>
<td>Weston-s-Mare</td>
<td>Rain</td>
<td>196; 180</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 2.17; TCAA concentrations of some natural aqueous samples**

*(Error range is standard deviation of n replicates)*
component. This has not been possible during this study, but future co-operation with other research groups using derivatisation techniques would be interesting.

![Graph](image.png)

**Figure 2.8; Intercomparison study between MoNKS distillation and HSGC**

*(Error bars are 95% confidence intervals on triplicate analyses)*

### 2.5 Conclusions

The MoNKS distillation has proved to be a reliable and accurate method for the determination of TCAA in a variety of environmental media. With the availability of large sample volumes/amounts the technique has a very low detection limit due to its high concentration factor, but is less powerful if the sample is limited. The usefulness of the technique for needles, rainwater and cloudwater is unquestionable, but there are problems with soil samples due to bumping and foaming.

The distillation must be performed with great care with respect to contamination. The conversion of TCAA to CHCl₃ at very low concentrations means that steps must be taken to avoid the presence of CHCl₃ in the laboratory. This is a particularly important consideration when working in an environment where solvents are widely used such as a chemistry department. The pre-heating of glassware and the regular monitoring of blank hexane minimises the risk of contamination.
An advantage of this method over HSGC is the ease with which the results may be calibrated. Simple external standards may be used to determine the concentration of CHCl₃ in the extracts. CHCl₃ may be routinely analysed in hexane extracts with any of the commercially available GC columns, due to simple chromatographic separation required and the lack of any interfering species.

Studies during this work have shown that efficient 100 % conversion of TCAA to CHCl₃ is possible with moderate temperatures within 1 hour, which has been proved using TCAA and CHCl₃ standards. The optimum MoNKS distillation parameters have been established and can be used as the basis for further work by this technique or the development of HSGC methodology. The use of different volumes of hexane (2 ml and 5 ml) to trap CHCl₃ has been proved to be efficient, which has led to reduced detection limits and the study of different media i.e. air samples. Degassing experiments have shown that relatively large amounts of CHCl₃ can be efficiently removed from samples prior to analysis so reducing the background interferences. This is particularly important for the analysis of real environmental samples that may have CHCl₃ adsorbed, which must be removed before TCAA determination. The scope of the degassing experiments were also useful for the development of a HSGC method (Chapter 3).

The monitoring of TCAA in needles by MoNKS distillation showed natural patterns that were of interest for further study. The tree-to-tree variation, even at bud burst, demonstrated the heterogeneity that is found at Glentress Forest before any influence of atmospheric deposition. The TCAA concentrations showed a maximum during the winter and a minimum in the summer, which is the reverse of observations in the literature. The variations measured in needle TCAA concentrations in year C needles over a growing season suggested that simple accumulation was not the only internal process at work and that metabolism of TCAA in needles was possible. It was suggested that the TCAA in needles was mainly located within the cells of the needle matrix rather than adsorbed on the surface, but surface-bound TCAA was significant in some cases.
The conclusions drawn from this limited data are the basis for further work at Glentress Forest and suggest areas that research might be focused on with more intensive sampling. The availability of analytical instruments meant that the continued use of this technique was difficult and led to the development of the automated HSGC technique. The preliminary results shown here were promising enough to win funding from Eurochlor for the purchase of an automated HSGC sampler, which was used for analysis of TCAA in the bulk of this thesis. The specialist equipment that is required for automated HSGC is expensive and relatively rare in a standard analytical laboratory and so the ability to perform analysis with a simple piece of glassware is a useful way of investigating TCAA in the environment. With a sufficient number of MoNKS distillation condensers it is possible to maintain a reasonably high throughput of samples, which more than matches that of the manual HSGC technique. The advantage of the MoNKS distillation is that the samples may be stored until the analytical equipment is available.
Chapter 3

The development of a Headspace Gas-Chromatography (HSGC) method for the analysis of TCAA in environmental samples

3.1 Introduction

There are several methods for the analysis of trichloroacetic acid (TCAA) at trace concentrations, which have been employed for its detection in environmental media. The most commonly used analytical technique for low molecular weight analytes such as TCAA, is Gas-Chromatography (GC), due to its high sensitivity. Because TCAA is insufficiently volatile the technique does not lend itself to such an analysis directly. To overcome this problem of involatility a variety of derivatisation procedures have been developed, which facilitate separation and detection of TCAA by GC (Frank et al. 1990; Ozawa & Tsukioka 1990; Frank et al. 1995). These methodologies mostly involve homogenisation of the matrix (e.g. needles) under liquid nitrogen followed by combination with water and centrifugation. The extraction of TCAA from its matrix into the aqueous phase is followed by acidification and partition into diethyl ether. An aliquot of the ether phase is then removed and derivatised with a reagent such as saturated diazomethane or 1-(pentafluorophenyl) diazoethane. The methyl ester derivative of trichloroacetic acid is then analysed directly by GC. The advantage with this method is the ability to measure not only TCAA, but also the homologous series of chloro-, bromo-, fluor- and mixed-halo acetic acids simultaneously. The increased interest in haloacetic acids (HAA) may warrant further research in atmospheric pollutants such as dichloroacetic acid (DCAA).

The possible problems that are associated with such a multi-step analytical procedure are numerous. The ability of the extraction procedure to transfer TCAA to the added
water is probably sufficient for matrices like needles, but may prove problematic for samples where binding may be strong or irreversible, such as soil. The addition of water, despite the fact that it may be doubly distilled, may lead to contamination from TCAA. It has been found during this research that even the use of high grade HPLC-grade water did not eliminate the presence of TCAA at concentrations of the order of 1-2 ng ml$^{-1}$, which if added to a sample with very low TCAA concentrations would cause the sample TCAA to be significantly altered. A final consideration of this method is the time-consuming nature of the initial purification of the diethyl ether and the dangers associated with using a reagent such as diazomethane. The ability to use less hazardous derivatisation reagents has improved with time, but the overall efficiencies associated with this multi-step derivatisation process means it is not a user friendly procedure, and can only be established with a great deal of time, training and facilities. The need for a more direct method with fewer steps and more automation allied with simpler sample preparation was essential for a more routine analysis of these important HAAs.

The most direct analytical technique is Ion Chromatography, which is suited to the fact that at most environmental pHs (> pH 1) TCAA is present as the trichloroacetate ion (TCA). Simple anions such as these are routinely determined by liquid chromatography and separation from interfering components such as phosphate is possible with the correct choice of column. Despite this, detection of trichloroacetate by conductivity detection is the limiting factor of the method. Frank et al. (1990) cited the lack of sensitivity as problematic and work in this thesis has enabled detection to concentrations as low as 10 μg l$^{-1}$, which is insufficient for the very low TCAA concentrations reported in soils and needles (Table 1.1). The ability to pre-concentrate trichloroacetate prior to analysis by Ion Chromatography (von Sydow et al. 1999 & 2000) is appealing, but in practice there have been problems with the recovery of TCA from the solid-phase. If a strong acid is used to elute the TCA then the associated anion, e.g. chloride if using HCl, is hugely overloaded in the chromatogram and can make the detection and quantitation of TCA impossible. For these reasons Ion Chromatography has seldom been used in the literature as the main analytical tool for TCAA determination.
The use of Headspace Gas-Chromatography (HSGC) as an alternative technique to the previous discussed methods is well described in the literature by Plüümacher & Renner (1993). The need for expensive equipment is limited, as a heating oven and a Gas-Chromatograph (GC) fitted with an electron capture detector (ECD) are the only prerequisites. The sample preparation is simple and can easily be performed in the field using pre-weighed glass vials, after which, only the weight or volume of sample must be determined. The analytical procedure is simple and can easily be performed manually as well as automatically, as described in Section 3.4.1 & 3.4.2. The ability to develop the HSGC method cheaply, without major chemical hazards allowed preliminary results to be obtained without the outlay of large sums of money for equipment and was the main reason for the use of this analytical technique in this project.

3.2 Theory of HSGC

HSGC is commonly applied to the analysis of volatile organic compounds (VOC) (Huber et al. 1988; Entz et al. 1982) and has been used widely for the determination of volatile chlorinated hydrocarbons since the end of the 1970s (Plüümacher & Renner 1993). The method is based on the equilibration of volatile compounds between the solid or liquid phase of the sample and the gas phase above it. In a closed headspace vial, the concentrations change at higher temperatures into a new equilibrium with higher concentrations in the gas phase. An aliquot of the headspace is then analysed by GC. If the conditions of temperature, sample and headspace volume and vial size are maintained then the partitioning between the sample and headspace remains constant leading to highly reproducible results. However, TCAA cannot be analysed directly by HSGC due to its low volatility and high water solubility. Therefore, the property of TCAA to undergo thermal decarboxylation to chloroform (CHCl₃) on heating is utilised (Køppen et al. 1988; Müller et al. 1974). The reaction proceeds quantitatively with moderate heating and enables the determination of both TCAA and VOCs in aqueous solutions (Renner & Mühlhausen 1989; Renner et al. 1990) and in a variety of tree foliage samples (Plüümacher & Renner 1993). The ability to convert TCAA to CHCl₃ enables detection using an ECD, which is exceptionally
sensitive to chlorinated species and allows the determination of very low concentrations of TCAA.

The main criticism of HSGC as a technique is the problem in distinguishing between background CHCl₃ present in the sample prior to decarboxylation and CHCl₃ produced after heating from the decarboxylation of TCAA. As it is only the total CHCl₃ concentration that is determined, the contribution from background CHCl₃ must be determined in order to give an accurate determination of CHCl₃ emanating from TCAA. This problem caused Frank et al. (1995) to describe the method as "less reliable" than the traditionally used derivatisation techniques, despite their associated efficiency considerations. However, Frank et al. (1990) also commented that HSGC is "less time-consuming and is preferable for routine analysis". This problem may be overcome by the analysis of a blank alongside decarboxylated samples during HSGC. Plümacher & Renner (1993) performed an injection of each vial after an hour of equilibration at 65 °C, which determined the contribution of background CHCl₃ in the sample. The vials were then decarboxylated and the total CHCl₃ analysed by HSGC. By subtraction of the initial CHCl₃ from the total CHCl₃, the TCAA in the sample was determined. As described in the method development section (Section 3.4), the background CHCl₃ value is often insignificant compared to the TCAA values determined, but has been measured throughout the project as a useful check for possible CHCl₃ contamination from neighbouring laboratories.

The procedure outlined by Plümacher & Renner (1993) was modified, but mostly on the basis of time efficiency. The decarboxylation procedure of heating sample vials for 72 hours at 65 °C (Frank et al. 1990; Plümacher & Renner 1993) was too time-consuming, so the procedure established was to decarboxylate at 100 °C for 90 minutes. The blank determination was performed on a replicate sample vial which was not heated at 100 °C, but instead equilibrated at 60°C prior to injection. Figure 3.1 shows the process occurring within a headspace vial during the determination of TCAA by HSGC.
Figure 3.1; A schematic showing the in-vial processes occurring during TCAA analysis by HSGC

3.3 Calibration and calculation of results by HSGC analysis

The calibration of the HSGC method is not simple in most cases, largely because of the complex variety of environmental matrices used for TCAA determination. Calibration using a simple set of external standards is not sufficient due to the possible differences in partitioning between aqueous standards and solid sample matrices. There may also be differences in the headspace volume between standard and sample vials, meaning that a substance-specific and a matrix-specific calibration is required. The calibration procedure used is a modified standard addition method, which is essential due to the unavailability of a TCAA-free matrix, and is described in detail by Plümacher & Renner (1993).

Ideally to determine the concentrations of TCAA in any matrix would require a standard addition procedure to be performed, and by extrapolation of the calibration line back to zero addition (x=0) would determine the TCAA concentration of the sample. However, this would mean preparing at least six standard addition vials per sample determination and would be very time consuming. If the gradient of the calibration line for TCAA in water (response factor) were determined at the same time as TCAA standard additions it would be apparent that the response factors of aqueous standards (f_w) and standard addition (f_a) were different, for the reasons mentioned above. However, if this procedure were repeated several times it would be
clear that the ratio of the response factors \((f_w / f_n)\) would be constant and independent of the actual daily detector sensitivity. This ratio is referred to as the partition ratio \((F_{wn})\). After the partition ratio is determined for a specific matrix, the TCAA concentrations can be determined in future analyses simply by determining the daily detector response factor using aqueous TCAA standards \((f_w)\). Equation 3.1 shows how the concentration of TCAA in a sample may be calculated from CHCl₃ peak areas and the determined partition ratio.

\[
C_n = \text{Area}_n \times f_w^{-1} \times F_{wn} \times m_n^{-1}
\]

*Equation 3.1*

where;

- \(C_n\) = concentration of TCAA in needles (ng g⁻¹)
- \(\text{Area}_n\) = peak area of TCAA in needles
- \(f_w\) = actual response factor of TCAA in water (area/ ng TCAA)
- \(F_{wn}\) = ratio of response factors [TCAA in water \((f_w)\) : TCAA in needles \((f_n)\)]
- \(m_n\) = mass of sample (g)

It must be noted at this point that the total peak area of CHCl₃ is a combination of CHCl₃ from decarboxylated TCAA and background CHCl₃ present in the sample. By subtraction of the peak area of the blank vial (60°C) from the peak area of the decarboxylated sample, the peak area due to TCAA in the sample can be determined.

The standard addition calibration involved the addition of a total of 1 ml of solution to the standard addition vials for two reasons. Firstly the vials were prepared by the addition of varying volumes of standard solution to a mass of sample, made up to 1 ml with water, to produce a set of standard addition calibration vials. The total volume of 1 ml was a volume that could be added reproducibly using auto-pipettes. Once a mean partition ratio had been determined for 1 g of sample and 1 ml of solution, those specific conditions had to be maintained for that partition ratio to be appropriate. Secondly, the presence of water in the samples was thought to give more even heating of the vial and thus more reproducible decarboxylation. Therefore this procedure was established for the determination of partition ratios by HSGC analysis.
However, further investigation identified an overestimation with the established protocol. The level of TCAA in the added water was non-zero, which using the above procedure was not corrected for. Therefore, in all analyses a set of four 1 ml water vials were prepared and analysed. By subtraction of the peak area of one water vial run as a blank (60°C) from the average of the areas of three vials run as samples (100°C), the concentration of TCAA in water was determined. The concentration of TCAA in water (HPLC, deionised, 18 MΩ, mineral) was never low enough to be ignored so a correction had to be made.

The total CHCl₃ determined in a headspace vial is a combination of contributions from TCAA in the sample, CHCl₃ in the sample, TCAA in added water and CHCl₃ in the added water. If the contributions from the different interferences can be minimised, as discussed in the individual experimental sections, then the TCAA in the sample can be more accurately determined.

Plumacher & Renner (1993) suggested that partition ratios varied by up to 14% from the mean of repeated determinations on the same matrix, which was acceptable to them due to the heterogeneity of the needles. It must be noted that in determining TCAA partition ratios Plumacher & Renner (1993) assumed that all TCAA would be converted quantitatively and reproducibly and so used CHCl₃ standard additions to derive the partition ratio and not TCAA. In reality an associated efficiency may be connected to the decarboxylation procedure and may lead to greater error about the mean partition ratio. Another very important point to note is that the determined partition ratios are very specific to the parameters used (vial size, thermostating temperature, sample mass etc.) so it is not possible to take partition ratios from the literature and apply them to a different system. Therefore, partition ratios must be derived for each matrix type and set of experimental conditions.
3.4 Method development

3.4.1 Manual HSGC method

3.4.1.1 Initial parameters

The manual analytical method for TCAA at trace concentrations in environmental media was based on published work by Køppen et al. (1988) and Plümacher & Renner (1993). Work performed in Edinburgh by Hansen (1997) and Henderson (1998) also yielded a background understanding of how a HSGC technique could be developed. This section describes the development steps taken to produce a reliable, robust analytical method.

The initial methodology closely followed parameters used by Frank et al. (1990) for the determination of TCAA by HSGC with the GC conditions based on a method for determination of CHCl₃ (Section 2.2.3). Amber headspace vials (30 ml) with screw cap tops fitted with PTFE/ rubber septa were used for analysis in this method. All vials were heated at 200°C prior to use and were stored sealed to prevent any contamination. Decarboxylation was achieved by heating at 100°C for 1 hour rather than the longer decarboxylation time at 65°C used by Plümacher & Renner (1993). This allowed a greater throughput of samples and when performing the method manually less time was spent waiting. The optimum decarboxylation time could be altered to give maximum TCAA conversion at a later stage. The vials were heated to decarboxylation temperature in a GC oven, which was controlled using a temperature programme, and then allowed to equilibrate for 2 hours at 60°C before injection. Blank vials for the determination of background CHCl₃ were prepared using the same procedure as the samples but were heated at 60°C. Aliquots of headspace (400 µl) were injected using a 500 µl Hamilton gas-tight syringe directly into the split injection port. To prevent any possible carry-over several pumps of the syringe were performed between injections as well as the determination of air blanks. Significant levels of CHCl₃ were not detected in either air blanks or subsequent injections. Initially a SE-54 capillary column (SGE) was used for analysis, but this was superseded by DB-624 columns (J&W) which produced excellent separation of
chlorinated hydrocarbons and meant that both HSGC and MoNKS methods (Chapter 2) used the same chromatographic conditions for the analysis of TCAA.

Initial results studied the reproducibility of injection of a 50 ppb TCAA standard. The RSD of three standards was 14%, which was not precise enough for such a trace analysis. The poor reproducibility was attributed to injection variability possibly caused by using a gas tight syringe at room temperature, which may have lead to condensation of the heated sample on the cold surfaces. However, the syringe could not be pre-heated as its accuracy would then be compromised. Another possible explanation was loss of analyte from the vial as the septum was pierced. Due to pressure build-up the septum often bulged after the heating period, which may have caused loss on sampling though this was never observed. These problems are associated with the manual use of this method, which would not be an issue if automated. To compensate for this injection variability an internal standard was considered the best approach to use.

3.4.1.2 Internal Standard

Iodomethane (CH₃I) was chosen as an appropriate internal standard for the development of this method. It was used for the same purpose in previous work (Brown 1995) and was ideal for this project as it has a low atmospheric occurrence and its retention time (RT) meant it did not co-elute with the peak of interest (RT CH₃I: 2.55 min; RT CHCl₃: 4.5 min). The iodine component also made it detectable by ECD.

When using an internal standard the most important factor is the precision with which it is added to each sample. For this reason 5 μl of a standard solution of 200 mg l⁻¹ CH₃I in methanol was added to each sample vial using a syringe fitted with a Chaney adapter to maximise precision. For three vials containing CH₃I at 60°C the RSD for injection was 2.8%. This sort of reproducibility is at the level required to routinely look for small differences in TCAA in the environment. It was also found that no carry-over of CH₃I occurred in either syringe or GC.
One area of concern about the use of CH₃I was its stability at 100°C. It was noted in previous development work that possibly in the presence of water at 100°C CH₃I was hydrolysed, which rendered it useless for this work. An experiment was designed to test this hypothesis. Three sets (A, B & C) of seven vials were prepared each containing 306 ng TCAA in aqueous solutions. The total volume in each set was 100μl (A), 1 ml (B) and 5 ml (C). To each vial 5 μl of 200 mg l⁻¹ CH₃I was added. Five vials in each set were decarboxylated (100°C for 1 hour) prior to equilibration (60°C for 2 hours), whereas 2 blank vials were heated at 60°C for 3 hours before all the vials were analysed by HSGC. Additionally a water vial of the appropriate volume for each set was analysed. The results are shown in Figure 3.2.

![Figure 3.2; CHCl₃ and CH₃I peak areas for Internal standard experiment](image)

*Figure 3.2; CHCl₃ and CH₃I peak areas for Internal standard experiment*

*(Bars represent mean ± standard deviation of duplicate analyses)*

The peak areas shown in Figure 3.2 indicate that CH₃I is undergoing some process of degradation after decarboxylation. The areas obtained for the internal standard for the blanks are very similar between each set of vials, allowing a slight decrease with increased water volume due to some differences in partitioning. However, the peak areas of the decarboxylated vials exhibit decreasing peak area with respect to their blank vials, which is more marked as the volume of aqueous solution increases. This
cannot be explained by simple partitioning as the blank vials which are heated at 60°C show only a small decrease in CH₃I peak area between 100µl and 5 ml of solution whereas in the sample vials there is greater than a 2-fold difference. The conclusion from this experiment is that CH₃I is degraded in the presence of water at 100°C and is therefore of no use as an internal standard.

On closer investigation the experiment also yields information on the reproducibility of TCAA injections without correction for an internal standard. The relative standard deviations obtained for 5 injections of each standard set (A, B & C) were 6, 1.2 and 2.1 % respectively. The precision of injections is excellent and proves that this manual procedure can be used for determination of TCAA without the need for an internal standard as was first thought.

3.4.1.3 Determination of decarboxylation efficiency

A recovery experiment was performed to determine whether the maximum peak areas achieved on decarboxylation of TCAA were reproducible and represented 100 % conversion to CHCl₃. This experiment produced two calibration lines, one from the decarboxylation of TCAA and another from CHCl₃ standards. Both sets of standards were prepared in the range 0 to 160 ng CHCl₃. When taking into account the relative masses of TCAA and CHCl₃ the gradients of the calibration lines should be the same. A constant volume of 100µl of solution was used in each set of vials. The TCAA vials were decarboxylated for 1 hour at 100°C before equilibration at 60°C for 2 hours, whereas the CHCl₃ vials were equilibrated for 3 hours at 60°C. The results from this experiment are shown in Figure 3.3.

The graph shows that conversion of TCAA to CHCl₃ is 98% complete and that the decarboxylation procedure quantitatively and reproducibly converts TCAA. After the experimental parameters were determined and optimised, the method was used for the analysis of samples for TCAA. The HSGC parameters established during this development procedure are shown in Table 3.1.
3.4.1.4 Determination of partition ratios for the manual HSGC method

When a reliable and robust HSGC protocol had been developed it was essential to determine the partition ratio for each environmental matrix and sample preparation that was used for routine analysis. The only partition ratio that was required for the manual method was for 0.5 g samples of ground needles with 1 ml of solution.

To determine the partition ratios for a particular matrix a standard calibration and a standard addition calibration to that matrix were performed as outlined in Section
3.3. Different stock standard solutions prepared at different times and different sets of needles (year class, sampling date and location) were used for a large number of standard addition determinations to produce a representative partition ratio that was not greatly affected by erroneous points. The use of freshly prepared standards and the natural needle variation ensured that the mean ratio and the error range was a good estimate of the true value of the partition ratio.

The preparation of standard additions was performed with Sitka spruce needles, which were ground to a powder with liquid nitrogen and well mixed prior to weighing. An accurate mass of 0.5 g (± 1 mg) of needles was transferred to a preheated headspace vial to which the appropriate volume of standard solution and water were added, so that the total volume of solution was 1 ml. Typically the standard range over which the partition ratios were determined was 0 to 100 ng TCAA. Each standard addition calibration graph usually contained seven data points of which one was a zero ng TCAA addition (1 ml water) and one was a blank vial. The blank vial was a normal preparation of standard/standard addition, which was heated at 60°C before injection. This value represented the CHCl₃ present in either the sample and/or water and was subtracted from the peak areas of the other vials to produce a calibration gradient relative to increasing TCAA without the interference of background chloroform. In practice the blank value was very often small. The zero vials (1 ml water) were important for the determination of TCAA in the needles. By extrapolation of the standard addition line (sample + TCAA + water) back to zero addition (x=0), the amount of TCAA in the needles can be estimated. However, because all the vials contain 1 ml of water, which may have a non-zero TCAA content, it must be corrected for.

The average water:needle partition ratio determined for the manual analysis of 0.5 g of ground Sitka Spruce needles was 1.10 ± 0.09. This value was determined on 9 standard addition calibrations and represented an 8% RSD. This value cannot be compared with the literature due to the different parameters used. A typical standard addition calibration graph is shown in Figure 3.4.
Definition of terms;

For standard calibration:
- Peak area = Total (TCAA + CHCl₃) in vial.
- Corrected peak area = TCAA added + TCAA in water (CHCl₃ subtracted).
- Intercept = TCAA in 1 ml water.
- Response factor = slope of line.

For standard addition calibration:
- Peak area = Total (TCAA + CHCl₃) in vial.
- Corrected peak area = TCAA added + TCAA in water + TCAA in needles.
- Intercept = TCAA in 0.5 g needles + TCAA in 1 ml water.
- Response factor = slope of line.

In this example (Figure 3.4)
- TCAA in needles = (145065 / 9576) - (84610 / 12359).
  = 8.3 ng in 0.5 g or 16.6 ng g⁻¹.
- Partition ratio = 12359 / 9576 = 1.29.
3.4.2 Automatic HSGC method

3.4.2.1 Theory

The manual method became obsolete (for most purposes) when an automatic HS40 XL headspace sampler was purchased from Perkin-Elmer. Whether the HSGC analysis of TCAA is performed manually or via an automated headspace sampler the underlying principles remain the same. The developed method outlined in Section 3.4.1 can be transferred directly to use on the automatic sampler with only a few alterations. In principle accuracy and precision should be improved by automation of the method as many of the sources of error mentioned in the previous section can be eliminated. In reality the complex nature of instrumentation requires much routine maintenance to optimise its operation, but provides the greatest advantage of automated analysis overnight leading to greater sample throughput. However, certain existing parameters and other new ones must either be changed or optimised for the automatic method for logistical reasons. For example, the injection needle is now heated so requires a certain temperature to be specified, and instead of an injection volume an injection time is required. This theory section outlines the mode of operation of the automatic sampler and any changes in the methodology from the manual method in Section 3.4.1.

The HS40 consists of a sample carousel, a vial heating system and an injection system, which can be simply interfaced to the PE Autosystem GC. The system operation is described below.

The carousel holds a maximum of 40 vials of 20 ml volume. If a missing vial is detected the sampler will move to the next vial position. The vial to be sampled is moved underneath the oven inlet and is transferred upwards into the oven. The oven may contain a maximum of 14 vials, which allows concurrent heating of vials. When the vial has been injected it is cooled and returned to the carousel.

The HS40 cannot perform both the decarboxylation and the equilibration steps before injection. The decarboxylation is still performed in a temperature programmed fan
oven. The time the vial is equilibrated for is now referred to as the *thermostatting time* and the temperature chosen is the *thermostatting temperature*.

To introduce the sample to the GC a heated needle is injected into the vial. The heated needle stops condensation of the analyte on it, but is only set at 10°C higher than the vial so as not to disrupt the established equilibrium. The injection system is not based on a syringe, but instead uses a set of valves which reduces the scope for error and is a patented feature of this system. On injection a valve switches and the needle starts to pressurise the vial with helium for a specified time, which is referred to as the *pressurisation time*. After this period the valves switch again and due to the higher pressure in the vial than in the GC some headspace sample is transferred along a *transfer line* to the GC injection port. The period which the valve remains open for is referred to as the *injection time*. To maintain the correct and reproducible operation of the injection system three rubber injection seals must be periodically changed. The transfer line is connected at one end to the needle and valve system and at the other to the GC. The line itself is a 1 metre section of fused silica capillary tubing with no phase attached, which is heated to stop condensation of the headspace gases. The *transfer line temperature* chosen must be higher than the boiling point of the analytes of interest, but may be maintained as high as desired, as the quantitative sampling of headspace has already been performed. The transfer line connects directly into the injection port and takes the place of the septum and injection nut. The GC start is instantaneously triggered by the HS40 when the injection valve opens. The whole system is controlled *via* a 600 series Interface. The signal is collected and produces a chromatogram using the PE Turbochrom Workstation software package.

A significantly different feature of the HSGC system compared to GC is how the carrier gas pressure is maintained through the column. The carrier gas is actually set using the HS40, which can now be thought of as an extension of the GC. A small head pressure (3 psi) is set using the GC pressure controls and then the required total carrier gas pressure is set using the pressure control on the HS40. The helium carrier gas therefore flows through the valve system on the HS40 and then through the GC.
Sample preparation also differs from that of the manual method. The vials used are 20 ml vials specific to the instrument. They are washed and heated at 200°C prior to use to remove CHCl₃ and TCAA. The vials are crimp-capped using a patented system consisting of an aluminium cap with hole, an aluminium star insert and a PTFE-coated butyl rubber septum. The star insert is used to stop the caps bursting if excess pressure builds up and allows higher temperatures to be used. The PTFE coating of the septa stops permeation of CHCl₃ while the rubber allows a self-sealing hole to be made.

3.4.2.2 Initial parameters

As previously discussed many of the parameters from the manual method can be used directly and certainly make a good starting point for the method development. All of the GC parameters remained the same, as the chromatography did not alter (see Table 3.1). Default injection parameters were mostly used, but the combination of the chosen injection time and split flow was designed to give appropriately sized peaks without broadening. The off-line decarboxylation time also remained 60 minutes at 100°C. The withdraw time specifies how long after injection the needle is removed from the vial and is important if there are very early eluting peaks, which is not the case here. The shaker agitates the samples during the thermostating time and maintains as complete equilibrium as possible.

<table>
<thead>
<tr>
<th>Thermostatting temperature</th>
<th>60°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermostatting time</td>
<td>60 min</td>
</tr>
<tr>
<td>Needle temperature</td>
<td>70°C</td>
</tr>
<tr>
<td>Transfer line temperature</td>
<td>90°C</td>
</tr>
<tr>
<td>Pressurisation time</td>
<td>2 min</td>
</tr>
<tr>
<td>Injection time</td>
<td>0.03 min</td>
</tr>
<tr>
<td>Withdraw time</td>
<td>0.00 min</td>
</tr>
<tr>
<td>Shaker</td>
<td>ON</td>
</tr>
<tr>
<td>Split flow</td>
<td>20 ml min⁻¹</td>
</tr>
</tbody>
</table>

Table 3.2; Initial automatic HSGC parameters
3.4.2.3 Method precision

The precision of the method was determined by the injection of multiple vials of TCAA standard solution. After decarboxylation at 100°C the vials were injected using the initial parameters. One experiment used 5 replicate vials containing 2.1 ng TCAA. The relative standard deviation achieved for injection was 1.9%. This is excellent precision for standards lying in the lower range of the method. However, typical RSD values are around 2-7% which is still very acceptable. The precision of the method is determined to a great extent by the integrity of the needle seals and may vary if the seals become damaged or need replacing. An integral part of the precision measurements are the decarboxylation efficiency and it is clearly shown to be very reproducibly.

3.4.2.4 Linear range

The linear range of the method has been determined many times when performing standard addition calibrations, but an experiment was designed to test the linearity at very low concentrations applicable to precipitation measurements. A set of 9 vials was prepared in the range 0 to 22 ng TCAA. The results are shown in Figure 3.5, 3.6 & 3.7. The TCAA calibration graphs are linear in the range 0 to 2.5 ng, 0 to 5 ng and 0 to 22 ng. However, it is interesting that the response factor for the first two ranges is identical (± 0.5%) whereas the response for 0 to 22 ng TCAA is slightly less (12%). This suggests that for samples that contain low TCAA concentrations, i.e. less than 5 ng per vial, should be calibrated with a standard no larger than 5 ng. This is important for precipitation samples where the concentrations may be approximately 1 ng per vial.
Figure 3.5; TCAA linearity in 0 – 2.5 ng range

\[ y = 48270x + 2959.3 \]
\[ R^2 = 0.9985 \]

Figure 3.6; TCAA linearity in 0 – 5 ng range

\[ y = 48011x + 3226.5 \]
\[ R^2 = 0.9996 \]
3.4.2.5 Optimisation of thermostatting time

To test whether equilibrating the vials for 60 minutes before injection was unnecessarily long an experiment was performed to test the effect of thermostatting times on standard peak area. Duplicate vials containing 26 ng TCAA were prepared and after decarboxylation for 60 minutes at 100°C were analysed by HSGC using thermostatting times between 5 and 60 minutes. The results are shown in Figure 3.8. The variation in peak areas between the different times were certainly no greater than between duplicate injections. The experiment suggests that a thermostatting time as small as 5 minutes could be used for equilibration but for safety the HSGC method used 10 minute equilibration times.

3.4.2.6 Optimisation of pressurisation time

An experiment was performed to test whether longer pressurisation times would increase the sensitivity of the method by increasing the amount of headspace transferred on injection. Duplicate vials containing 26 ng TCAA were prepared and after decarboxylation for 60 minutes were analysed by HSGC using pressurisation times varying between 1 and 4 minutes. The results are shown in Figure 3.9.
Figure 3.8; The effect of thermostatting time on peak area

(Error bars are the range of duplicate determinations)

Figure 3.9; The effect of pressurisation time on peak area

(Error bars are the range of duplicate determinations)
The pressurisation time has very little effect on the peak areas of the standards. The apparent optimum pressurisation time for HSGC analysis is 2 or 3 minutes which agrees with the value chosen initially.

3.4.2.7 Optimisation of decarboxylation time

A further test of the optimum decarboxylation time established in Section 3.4.1.3 was performed. Three separate experiments were performed testing decarboxylation times between 30 and 120 minutes. Duplicate vials containing 26 ng TCAA were prepared and analysed by HSGC. The results are shown in Table 3.3.

<table>
<thead>
<tr>
<th>Decarboxylation time (minutes)</th>
<th>Peak area Expt. 1</th>
<th>Peak area Expt. 2</th>
<th>Peak area Expt. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>963934 ± 174464</td>
<td></td>
<td></td>
</tr>
<tr>
<td>58</td>
<td></td>
<td>1476549 ± 73323</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1523095 ± 29579</td>
<td>1531082 ± 196062</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>1722852 ± 23981</td>
<td></td>
<td>1661679 ± 54206</td>
</tr>
<tr>
<td>95</td>
<td></td>
<td></td>
<td>1648803 ± 45959</td>
</tr>
<tr>
<td>120</td>
<td>1778454 ± 73231</td>
<td>1687860 ± 115852</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3; The effect of decarboxylation time on peak areas

*(Error bars are standard deviations of duplicate samples)*

The peak areas from experiment 1 indicated that a decarboxylation time greater than 60 minutes produced a significantly higher peak area, which was unexpected so was investigated further. It was clear that a 30 minute decarboxylation time did not give a full conversion of TCAA and was not repeated. Experiment 2 suggested there was no significant difference between the three decarboxylation times, but that it was possible that full conversion was achieved within 90 minutes. This was confirmed in experiment 3 where decarboxylation times longer than 90 minutes produced no further increase in peak area. It must be noted that though the areas achieved for 60 minutes were similar to those for 90 minutes, the extra 30 minutes heating produced an extra 10% peak area on average.
3.4.2.8 Established parameters for automatic HSGC method

After the optimisation procedure outlined above the parameters in Table 3.4 were established for use in the determination of the partition ratios and for routine analysis of the various matrices for TCAA.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decarboxylation time</td>
<td>90 min</td>
</tr>
<tr>
<td>Decarboxylation temperature</td>
<td>100°C</td>
</tr>
<tr>
<td>Thermostatting time</td>
<td>10 min</td>
</tr>
<tr>
<td>Thermostatting temperature</td>
<td>60°C</td>
</tr>
<tr>
<td>Needle temperature</td>
<td>70°C</td>
</tr>
<tr>
<td>Pressurisation time</td>
<td>2 min</td>
</tr>
<tr>
<td>Injection time</td>
<td>0.03 min</td>
</tr>
<tr>
<td>Transfer line temperature</td>
<td>200°C</td>
</tr>
<tr>
<td>Split flow</td>
<td>20 ml min⁻¹</td>
</tr>
<tr>
<td>Injector temperature</td>
<td>200°C</td>
</tr>
<tr>
<td>Detector temperature</td>
<td>375°C</td>
</tr>
<tr>
<td>Oven temperature (Initial; Final)</td>
<td>50°C; 150°C</td>
</tr>
<tr>
<td>Time (Initial; Final)</td>
<td>6 min; 3 min</td>
</tr>
<tr>
<td>Rate</td>
<td>25°C min⁻¹</td>
</tr>
<tr>
<td>Carrier pressure (He)</td>
<td>12.6 psi</td>
</tr>
</tbody>
</table>

Table 3.4: The established HS40 method parameters

3.4.2.9 Determination of partition ratios for an automatic HSGC method

The determination of partition ratios using the automated technique used the same procedure as the manual technique as outlined in Section 3.3. Using the parameters listed in Table 3.4 the partition ratio for soil and needles were determined for 0.5 g and 1.0 g fresh weight of sample. Note that in preparing the vials 1.0 g ± 1 mg were weighed. Soil vials were prepared in a similar way except that before weighing, the soil was sieved using a 2 mm mesh and well mixed. For the same reasons as previously discussed with needles, soils of different types and sampling date were used to give a representative partition ratio. The partition ratios determined are shown in Table 3.5.
\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
Mass of sample & Sitka Spruce needles & Soil \\
\hline
0.5 g & 1.26 ± 0.16 & 1.21 ± 0.23 \\
& (n=9) & (n=11) \\
1.0 g & 1.94 ± 0.26 & 1.98 ± 0.16 \\
& (n=7) & (n=7) \\
\hline
\end{tabular}
\caption{Partition ratios for the automatic HSGC method}
\end{table}

(Error range represents standard deviation of n determinations)

The striking feature about the ratios shown in Table 3.5 is that the magnitude of the value appears independent of the matrix analysed and seems to be more influenced by the mass of the sample. Another expression of this would be that the partitioning for 1 g of soil and needles are the same. This is unlikely unless CHCl₃ is fully partitioning into the headspace, as the amount of organic matter in the soil compared to the needles would be vastly different. Therefore it suggests that the partition ratios are primarily dictated by the volume of headspace into which the CHCl₃ is concentrated. The differences in total organic matter, density and the partitioning between sample and headspace in needles and soil are relatively minor factors compared to the volume of the headspace in 20 ml vials, which is shown by the reproducibility of the determined partition ratios. The standard deviation of the mean is low, especially when considering the variability between the different soil and needle samples used for the standard addition calibrations.

The determination of these partition ratios allows the continued direct analysis of environmental matrices without the need to perform standard addition calibrations. If the effect of sample type on partition ratio could be elucidated then a more generic volume/ mass based ratio could be employed to all types of sample. If a relationship between sample mass/ volume and partition ratio can be determined any new sample type i.e. bark, could be analysed without the need to perform a set of matrix-specific standard additions. It is yet to be demonstrated whether the partitioning with needles and soil are coincidentally the same or whether partitioning is negligible for both matrices meaning differences in headspace volume are dominant in determining the partition ratio.
Chapter 4

Trichloroacetic acid in Sitka Spruce needles

4.1 Introduction

The presence of trichloroacetic acid (TCAA) in the environment was first investigated after the discovery of TCAA in needles of coniferous trees in remote areas (Frank et al. 1989). There were no direct sources of TCAA so its occurrence was thought to be due to atmospheric input, either by the direct uptake of chlorinated solvents by the trees and its subsequent in situ conversion to TCAA or by formation of TCAA in the atmosphere and its uptake in wet deposition by trees. The phytotoxic properties of TCAA and the other chlorinated acetic acids meant that they were implicated as a stressor in the "precocious loss of conifer needles" in Finnish and Central European forests (Frank et al. 1992). The signs of needle discoloration and loss may be more obviously seen in Central Europe due to a lack of minerals in trees, particularly magnesium, not experienced in Great Britain due to its input from sea salt (Roberts et al. 1989). Frank (1991) admitted that other chemicals such as sulphur dioxide, ozone and the oxides of nitrogen as well as pentachlorophenol and nitrophenols may also have an effect. In reality it is probably the combined synergistic effect of these compounds as well as the interaction with climate and the management of forests that is likely to be responsible for the observed forest decline.

After irradiation with strong sunlight, trichloroethene and tetrachloroethene, possible TCAA precursors, have been shown to negatively affect conifers at concentrations about ten-fold higher than ambient by degrading the trees' photosynthetic pigments, particularly chlorophyll a (Frank & Frank 1985; Figge 1990; Frank 1991). High TCAA concentrations have also been shown to affect photosynthesis (Sutinen et al. 1995) and to decrease growth (Sutinen et al. 1997). However, as noted by Frank et al. (1990) nothing is known about the chronic effects of continuous exposure of forest trees to low levels of TCAA.
Several monitoring campaigns have been performed measuring the concentrations of TCAA in needles of different species of tree across Europe. A database of 600 analyses determined TCAA throughout Europe in the range 5 to 130 ng (g fwt)$^{-1}$ TCAA (Norokorpi & Frank 1995) and a similar exercise by Juuti (1997) with a sample size of 1600 determinations from 7 authors gave a similar concentration range of 0.6 to 178 ng (g fwt)$^{-1}$ TCAA. The limitation of using a range of needle concentrations determined using different analytical methodologies, in samples from different sites, countries, tree species, needle year class etc., is that the differences and trends which may be caused by specific conditions may be hidden amongst a generalised group. The trends that have been recognised are well documented and discussed later. The large range of concentrations found demonstrates that TCAA is present in all samples in trace amounts, but may vary according to different climatic and growing conditions, an effect which must be elucidated.

The link between the damage observed in trees and the needle TCAA concentrations is yet to be proved conclusively. The results reported by Norokorpi & Frank (1995) revealed a correlation between the degree of defoliation and the TCAA content of needles, but later work at the same site did not show a statistically significant dependence of visual tree vitality parameters on TCAA concentrations. This illustrates the difficulties in using subjective parameters such as degree of defoliation and tree vitality, as well as the problems encountered when working at environmental concentrations in natural systems such as forests.

Bold predictions about the trends that should be observed were stated in the original work by Frank et al. (1990), which suggested that TCAA concentrations were always higher in older needles and that there was a distinct seasonal variation of TCAA during the year. A similarly bold assessment of the available data was made by Sinkkonen et al. (1998), which claimed that TCAA had been found to bioaccumulate in conifer needles, citing work by Juuti et al. (1993) and Frank et al. (1992) as evidence. These claims overstate the issue because if seasonal maxima are caused by the ability of trees to degrade TCAA at different rates during the year to produce a
seasonal variation then bioaccumulation does not seem possible, rather the maintenance of steady state concentrations.

The variations of TCAA in needles observed by some authors (Frank et al. 1994; Plümacher 1995) could be explained by two different formation theories. Firstly a maximum in solar intensity and temperature could lead to increased production of atmospheric TCAA due to the dependence of its formation reaction on photolytic and chemical reactions, and so would lead to increased TCAA input to trees in summer. Secondly, if TCAA is produced within trees after uptake/partition of the chlorinated precursors into needles, then this process may also peak in the summer months when the solar intensity would be strong enough to give conversion to TCAA. Evidence for the in situ production of TCAA from these precursors is not widespread. Work by Juuti et al. (1996) found no statistically significant differences in TCAA concentrations within a stand of trees between those receiving the highest (edge) and the lowest (centre) solar radiation. The higher concentrations of TCAA theoretically produced in the atmosphere during the summer months may also coincide with lower wet input, so would lead to the observation of higher concentrations but similar total TCAA input. An increase in TCAA with year class of needles has also been observed by Plümacher & Schröder (1994). This apparent accumulation is in direct contrast to the idea of a seasonal variation, with lowest concentrations during winter. If accumulation was the sole process occurring then, irrespective of varying TCAA input, the concentrations would increase continuously. The absence of constantly increasing needle TCAA concentrations, and also the lack of any clear correlation between needle loss and their TCAA concentrations suggests that a process of metabolism is counter-acting the continual uptake of this xenobiotic compound. This has been accepted as one explanation by Frank et al. (1992) and Plümacher & Schröder (1994), but has not been proved.

The idea of bioaccumulation is appealing for many authors and it has been suggested that TCAA could be used as an indicator for the distribution and deposition of phytotoxic photooxidants derived from the anthropogenic C₂-chlorocarbons (Norokorpi & Frank 1994). If internal metabolic processes are proved then the
theory that trees act as historical records for TCAA pollution events cannot be valid, as the concentrations would then represent a steady-state between the uptake and the degradation processes. The fact that trees maintain TCAA concentrations below a certain level may mean it has no direct harmful effect and that physiological damage is caused only if very high TCAA concentration events occurred or if the metabolism was somehow overloaded. However, this hypothesis must be tested by controlled seedling experiments (Chapter 7).

No significant variations in needle TCAA concentrations have been reported in the literature with the branch height on a tree (Frank et al. 1992) nor between regions in Finland (Juuti et al. 1996). This is surprising as differences in needle concentrations would be expected depending on the atmospheric input of TCAA, which could vary according to the climatic conditions from region to region or at different canopy heights within a stand. The spatial variability and seasonal trends observed and the mechanisms that control them have been described in the literature after a number of European sampling campaigns. The aim of this research was to perform an intensive spatial and temporal sampling programme at a site in Great Britain to compare and contrast the findings with previous measurements and hypotheses about the origin and uptake of TCAA in forests.

4.2 Sampling site

The site studied during this particular research is Glentress Forest, situated in the Southern Uplands of Scotland, 19 km south of Edinburgh (Figure 4.1). It is situated on a hillside varying in altitude from 225 metres above sea level (m asl) to 602 m asl at the highest point. Glentress Forest is a mixed forest, but mostly contains Scots Pine (*Pinus sylvestris* L.), Norway Spruce (*Picea abies* L. {Karst}), Sitka Spruce (*Picea sitchensis* {Bong.} Carr.), Douglas Fir (*Pseudotsuga menziesii* {Marb.} Franco) and Larch (*Larix decidua* Mill.). Sitka spruce grow at all altitudes throughout the forest and therefore was the species chosen for this study. Glentress Forest is a planted forest originating from 1900, but mostly dating to the 1950s. The trees were planted by hand after ploughing a furrow so disruption in the soil profile
Figure 4.1: A map showing Glentress Forest near Peebles, Scotland
may be observed. Forest management means that stands of trees of different ages exist throughout the forest.

The investigation of the routes of uptake of TCAA from the atmosphere were performed at two sites, which were intensively studied by this research. The criteria for the site selection were that the two sites should be at different altitudes to ensure that the higher site was exposed to the direct impaction of cloudwater and had a marked increase in wet deposition. Both sites should also possess vegetation of the same species and stature.

The upper site (elevation 602 m) was established near to a radio station at Dunslair Heights (3°8'W 55°41'N) situated on open area of moor land between an established mixed conifer forest (46 years old) and a younger Sitka spruce plantation (36 years old). It has good accessibility by track and is used for other monitoring campaigns by the Centre for Ecology and Hydrology (CEH). A meteorological station is operated at Dunslair Heights where long term measurements of cloud frequency and the chemical composition of wet deposition are made, as well as ozone measurements as part of the Netcen. Ozone Monitoring Network. A full description of the station is given by Crossley et al. (1992 & 1998). The lower site (elevation 275 m) is situated at Venlaw on a contour track in the middle of mixed-age plantation blocks (south of Whitfold Hill on Figure 4.1), which is used by CEH for rainwater collection only. The planting density is less at Venlaw because the standard spacing has increased since that site was established about 15-20 years ago. There is a distinct wet deposition gradient between the two sites with 1150 mm wet deposition at Dunslair Heights and 960 mm at Venlaw during 1999 (Chapter 6). If smaller needle TCAA concentrations were observed at Venlaw compared to Dunslair Heights it would suggest that wet deposition via rain and cloud dominated, whereas if similar concentrations were found at the two sites then input of TCAA in the gas-phase might be implicated.
4.3 Sampling programme

Sampling of Sitka spruce needles was performed at Glentress Forest between October 1998 and April 2000. Samples were taken prior to this date, but were primarily used for method development or for analysis by MoNKS distillation (Chapter 2). The data produced during this sampling period was used to monitor any seasonal patterns in TCAA concentrations using HSGC. The sampling was carried out at approximately monthly intervals.

To investigate the accumulation of TCAA over time as many year classes of needles as possible were sampled. The number of year classes was limited by the age of the trees in the stand, their health and planting density. Coniferous trees drop senescent needles, which are a net energy consumer and provide no net photosynthetic gain to the tree, so it was not possible to sample greater than 6 year classes of needles at Dunsclair Heights (current \(C\) to 6 year old \(C+5\)) and greater than 4 at Venlaw. In some cases older year classes have been analysed and the results are shown, but the sample sizes are small and are not likely to be representative.

During each sampling trip 5 branches were sampled from trees in different stands at each site using "squirrel" extending pruners. These branches were large enough to contain the appropriate number of needle year classes for each site and therefore contained a large number younger needle shoots. A total of 5 branches were taken to get a representative sample for each site. The branches were taken to the laboratory and the shoots of needles were removed, rinsed with water and separated according to their year class. The needles from each year class were removed quickly and easily by immersing in liquid nitrogen. The needles of each year class from the 5 branches were combined to give a pooled sample. The pooled needle samples were kept frozen until analysis was performed.

Some other non-routine sampling was carried out as part of this research. During May and June 1999 sampling of needles was carried out on a weekly basis. The current year needles that appeared during this period of bud burst were sampled to investigate the trend in TCAA concentration during bud opening and needle
elongation. During routine sampling on 27/10/99 not only the needles were sampled and stored, but also the stem material. The stem material was characterised according to age and later analysed for TCAA.

4.4 Experimental

4.4.1 HSGC analysis

Needles were analysed by automatic HSGC using the conditions outlined in Section 3.2.4.8. The needles were removed from freezing and dried using tissue paper to remove any surface moisture. A bulk sample was weighed (6 g) and the needles were ground under liquid nitrogen using a pestle and mortar. The powder was allowed to equilibrate at room temperature and was well mixed before 1.000 g (± 0.001 g) of sample was transferred to glass headspace vials previously heated and cooled to drive off any CHCl₃. To these vials 1 ml of HPLC-grade water was added and the vials were sealed with a PTFE coated rubber septum. The water added to the vials had been degassed using oxygen free nitrogen (OFN) for 1 hour to remove any CHCl₃ present, which minimised the variability caused from having high blank values in the analytical method. Vials were prepared in quadruplicate to allow the analysis of 3 sample TCAA and 1 CHCl₃ blank determinations. Unless the samples were to be analysed immediately the prepared vials were kept in a freezer to stop any possible breakdown of analyte.

In order to determine the TCAA concentrations the vials were put through the procedure of decarboxylation and HSGC analysis using the conditions shown in Table 3.4. The blank vials were heated at 60°C to determine background CHCl₃ in the samples.

During HSGC analysis the needle samples were run in a sequence between 2 sets of TCAA standard calibration vials. These vials were used to calculate the response factor to TCAA throughout the analysis. The TCAA standards were analysed every 8 injections and consisted of a standard vial, prepared prior to each set of analyses, containing a known mass of TCAA in 1 ml of solution and a water vial containing
1 ml of the water used to prepare the stock standard with. The CHCl₃ peak areas of the standard and water vials were used to calculate the response factor according to Equation 4.1.

\[
RF_{\text{TCAA}} = \left( \frac{\text{peak area (standard)}}{\text{peak area (water)}} \right) \quad \text{Equation 4.1}
\]

The measured sample peak areas were used to calculate the TCAA present in the samples according to Equation 3.1 (in Section 3.3) using the partition ratio of 1.94 reported in Section 3.4.2.9 to correct for the calibration with 1 ml aqueous standards. A set of 4 vials, each containing 1 ml of the water which had been added to the needle vials, were analysed by the same procedure as the needle vials (1 blank and 3 samples). This determined the concentration of TCAA in the water and was used for calculation of results (see Section 3.3). The analysis of the stem material from 27/10/99 was prepared and analysed according to the same procedure as above and the needle partition ratio was used to quantify the data.

4.4.2 Loss on drying

The needle water content can show a large variation depending on the maturity of the needle and the year class. For example, the water content of freshly flushed needles was approximately 80% of fresh weight, whereas older (C+5) needles contained as little as 40% water. This variation means that when weighing 1 g of sample there are varying amounts of needle material in the vials, depending on the age of the sample. In order to make a valid comparison, and elucidate appropriate trends with needle age, TCAA concentrations were expressed in terms of the needle dry weight.

Needle dry weight was determined by drying a known mass of needle material until constant weight was achieved. A low temperature was used so that no breakdown of material was likely. The procedure was to weigh fresh material into a pre-heated glass vial and then to heat at 60°C for 4 days. This time period was shown to yield no further weight loss.
Although the dry weight of a needle sample can be determined as above, the appropriate fresh weight (for comparison with previously published data) is less straightforward, because of the different processes used to store and prepare needle samples for analysis. After the needles were sampled from the forest they were removed using liquid nitrogen. The needles were then stored frozen for as long as a year. During the preparation of the vials for analysis the needles were ground with liquid nitrogen. An experiment was performed to compare the water content of the whole needles after sampling with the ground samples, which were later weighed into the vials for analysis. The results are shown in Table 4.1. The average of the differences between the losses is only 0.7%. It appears that liquid nitrogen does not ‘freeze dry’ the needle samples. The explanation for a slightly higher result for the ground samples may be that the water can be more easily lost from the ground samples than the whole ones. However, the variation between duplicate losses on drying of whole needles has been determined to be between ±0.2% and ±0.8%, suggesting that the differences in Table 4.1 are not significant. For these reasons the dry weights reported here refer to measurements from ground samples.

<table>
<thead>
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<th>Sample ID</th>
<th>Whole needle</th>
<th>Ground needle</th>
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</tr>
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<td>D3</td>
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</tr>
<tr>
<td>D4</td>
<td>50.7</td>
<td>51.7</td>
<td>+1.0</td>
</tr>
<tr>
<td>D5</td>
<td>58.9</td>
<td>57.2</td>
<td>-1.7</td>
</tr>
<tr>
<td>V1</td>
<td>44.6</td>
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<td>49.5</td>
<td>+1.4</td>
</tr>
<tr>
<td>V3</td>
<td>53.4</td>
<td>53.2</td>
<td>-0.2</td>
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<tr>
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<td>61.3</td>
<td>+2.1</td>
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<td>+1.4</td>
</tr>
<tr>
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<td>48.3</td>
<td>+0.5</td>
</tr>
<tr>
<td>W3</td>
<td>50.7</td>
<td>51.0</td>
<td>+0.3</td>
</tr>
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<td>W5</td>
<td>56.0</td>
<td>56.3</td>
<td>+0.3</td>
</tr>
</tbody>
</table>

Table 4.1; Percentage water content of ground and whole needles
(Each value is the mean of duplicate determinations)
4.5 Results and discussion

4.5.1 Variations in TCAA at bud burst

Figure 4.2 and 4.3 show the temporal patterns in TCAA concentrations in current year needles at Glentress Forest during May and June 1999.

Figure 4.2; The temporal pattern of TCAA in current year needles during bud burst at Dunslair Heights
(Error bars are standard deviations on triplicate analyses)

Figure 4.3; The temporal trend in TCAA in current year needles during bud burst at Venlaw
(Error bars are standard deviations on triplicate analyses)
As shown in Figure 4.2 and 4.3 the concentration of TCAA present in the flushing needles at bud burst varies considerably with time and between the two sites. Needles emerged from their buds later at Dunslair Heights than at Venlaw, as expected at a higher altitude, primarily due to the temperature difference between the two sites. There is little evidence of a temporal pattern at Dunslair Heights (Figure 4.2). In the first week the needles contained approximately 20 ng (g dwt)$^{-1}$ TCAA, but a week later that concentration had risen to 55 ng (g dwt)$^{-1}$ TCAA. By the following week the TCAA had dropped significantly and remained less than 20 ng (g dwt)$^{-1}$ TCAA for the next month. It is not clear whether the large value on 26/5/99 was due to high input for that week or to some other factor. The input of TCAA from rain during the week prior to 26/5/99 was the lowest for the month of May, but the cloud input was 3.84 µg m$^{-2}$, which was higher than the weekly average for that month (mean = 2.68 µg m$^{-2}$ wk$^{-1}$). However, the input was much higher for the week ending 9/6/99, with a 3-fold higher input from rain (see Chapter 6), but this did not result in a subsequent observation of an increased needle TCAA concentration. It is unclear if the fall in concentration in the week following 26/5/99 was caused by metabolism of TCAA or if a dilution occurred due to the elongation of the needles.

There is a temporal pattern apparent in the Venlaw TCAA concentrations (Figure 4.3). The needles collected on 12/5/99 were sampled at least a week after flushing, suggesting that the lower initial value observed in Dunslair Heights needles may have been missed at Venlaw. The initial value of 50 ng (g dwt)$^{-1}$ TCAA is in good agreement with the highest values at Dunslair Heights and was maintained for three weeks. This suggests that during this period the TCAA was neither metabolised nor was it diluted by the extension of the needles. It is more likely that the uptake of TCAA was equal to the dilution experienced due to needle growth. The high concentration then quickly fell to 20 ng (g dwt)$^{-1}$ TCAA which remained constant for another 3 weeks. The change may be due to a sudden increase in needle extension and the subsequent dilution of the TCAA concentration. Although measurements of needle length were not taken, the water content of the needles was determined each week (Table 4.2) and may be useful as a measurement of growth dilution.
The results in Table 4.2 show that the water content of the needles at Venlaw decreased with time as they expanded. A large drop in water content occurred during the week ending 2/6/99 and may represent a sudden spurt in growth or elongation of the needles. This correlates well with the observed pattern in TCAA concentrations. The large drop in water content between June and July may represent other factors apart from growth, such as surface wax formation. The pattern in water content is not as uniform at Dunslair Heights and is more complicated, showing increases and decreases over the period until a similar water content at both sites was achieved in July. This may explain the lack of a pattern in TCAA values at this site.

Needles expand fairly rapidly after bud burst for about 1 month and then stop. Subsequent increases in needle weight occur because of accumulation of fixed carbon (often starch) in the needles. This process is obvious in the TCAA pattern at Venlaw where a constant needle TCAA concentration is reached within 1 month of the bud burst. The phenomenon is well known and has been reported for other analytes (Linder & Flower-Ellis 1989), but why it is less obvious at Dunslair Heights is unclear. However, the additional factor of cloud events at this site may complicate the pattern. When the needles and stems are immature the direct uptake of TCAA or other solutes may occur (see Chapter 7), and so the effect at Dunslair Heights, where there are many cloud events sometimes with enriched concentrations, would be greater than at Venlaw where no cloud is experienced (Crossley et al. 1992). Allied to this is the reported higher concentration of ions in cloud compared to rain (Crossley et al. 1992). This is the first work to report the dilution effect for TCAA during flushing and was only noticed because of the time resolution of the study.

### 4.5.2 Temporal pattern of TCAA at Glentress Forest

The results shown in Figure 4.4 and 4.5 are the TCAA patterns at the two sampling sites in Glentress Forest for the 1998 growing season and in Figure 4.6 and 4.7 for

<table>
<thead>
<tr>
<th>Date</th>
<th>Venlaw</th>
<th>Dunslair</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/5/99</td>
<td>82.2</td>
<td>76.5</td>
</tr>
<tr>
<td>19/5/99</td>
<td>81.0</td>
<td>80.3</td>
</tr>
<tr>
<td>26/5/99</td>
<td>80.5</td>
<td>79.8</td>
</tr>
<tr>
<td>2/6/99</td>
<td>75.2</td>
<td>81.4</td>
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<td>9/6/99</td>
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<td>23/6/99</td>
<td>70.2</td>
<td>63.8</td>
</tr>
<tr>
<td>28/7/99</td>
<td>62.4</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2: Variation in water content (% fresh wt.) in Year C needles at two sites in Glentress Forest between May and July 1999
the 1999 growing season. The results are reported as nanograms of TCAA per gram dry weight of sample \([\text{ng (g dwt)}^{-1} \text{TCAA}]\), and the error bars are standard deviations of three replicate samples.

One aim of the project was to determine whether certain phenomena found in Europe such as bioaccumulation, summer TCAA maxima and increasing TCAA with altitude occurred at the Glentress Forest field site in Southern Scotland. The design of the sampling programme meant that these hypotheses could be objectively tested. If the sampling programme had utilised a ‘snapshot’ approach, measuring TCAA concentrations in different ages of needles only once during the year, it can be seen from Figure 4.4 that the results could yield erroneous conclusions. For example, the results obtained on 8/9/99 would suggest that TCAA accumulates with year class, implying a constant uptake of TCAA. However, this is not the case. TCAA concentrations have been found to increase and decrease throughout the year and this would suggest that simple accumulation of TCAA is not the explanation.

Figure 4.8, 4.9 & 4.10 replot the data from the earlier figures to show the changes in TCAA concentrations at Dunslair Heights and Venlaw in the 1998 and 1996 year class of needles between October 1998 and April 2000. The pattern in 1998 needles does not show an accumulation of TCAA with time. At Dunslair Heights there appears to be a cyclical change in TCAA. The maxima occurred in the winter months of 1998 and 1999 with concentrations about 3 times higher than during the summer. Conversely there was a minimum in summer 1999 and another decline was already apparent in the spring of 2000. The same pattern was also observed in the 1996 needles suggesting the processes responsible still occurred in older needles (Figure 4.10). These findings are contrary to the maxima observed during summers in the Northern Black Forest, Germany (Frank et al. 1990). The results at Venlaw (Figure 4.9) did not show as clear a pattern as Dunslair Heights. The concentrations were very similar from month to month over the whole time period and no accumulation of TCAA was noted. A smaller increase in TCAA occurred during both winters, which then decreased towards the spring in a similar pattern to that at Dunslair Heights.
Figure 4.4; Temporal pattern in needle TCAA concentrations at Dunslair Heights during 1998 growing season

(Error bars are standard deviations on triplicate analyses)

Figure 4.5; Temporal pattern in needle TCAA concentrations at Venlaw during 1998 growing season

(Error bars are standard deviations on triplicate analyses)
Figure 4.6; Temporal pattern in needle TCAA concentrations at Dunslair Heights during 1999 growing season

(Error bars are standard deviations on triplicate analyses)

Figure 4.7; Temporal pattern in needle TCAA concentrations at Venlaw during 1999 growing season

(Error bars are standard deviations on triplicate analyses)
Figure 4.8; Temporal pattern of TCAA concentrations in current year (1998) needles at Dunslair Heights

(Error bars are the standard deviation of triplicate analyses)

Figure 4.9; Temporal pattern of TCAA concentrations in current year (1998) needles at Venlaw

(Error bars are the standard deviation of triplicate analyses)
4.5.2.1 Discussion of results

If TCAA is not accumulated with time what are the possible reasons for the observed patterns? The input of TCAA from rain and cloud occurs constantly throughout the year (see Chapter 6) and as yet no TCAA-free precipitation has been observed. Trees lose water due to transpiration throughout the year and so consequently TCAA is continually taken up from roots via the transpiration stream. This suggests that TCAA must be metabolised within the trees to maintain the concentrations observed. Metabolic processes must cause the decreases or minima observed in the TCAA concentrations. This idea is now widely accepted (Frank et al. 1991; Plümacher 1995). The pattern at Dunslair Heights suggests that TCAA begins to accumulate during the winter months, implying that there is an ineffective or inhibited metabolic removal process. There could be several reasons to explain this. Firstly the colder temperatures at the more elevated Dunslair Heights site could ‘switch off’ metabolism during winter leading to a gradual accumulation of TCAA, which would then be degraded in the spring as the temperature increased and metabolism resumed. The accumulation of TCAA occurs at both sites during winter, but starts later at
Venlaw (October) than at Dunslair Heights (September), which may be influenced by the difference in temperature between the two sites. Alternatively, the input of TCAA at Dunslair Heights may be so much greater than at Venlaw that the metabolism is overloaded, resulting in an increase in needle concentration. However, the input of TCAA by wet deposition to the forest at Dunslair Heights was determined to be 50% higher than at Venlaw, which would not support this theory (1000 µg m⁻² yr⁻¹ compared to 675 µg m⁻² yr⁻¹ during 1999 growing season). The reason for increased needle TCAA concentrations is more likely to be related to changes in TCAA metabolism within the trees rather than large increases in uptake, as the trees transpire considerably less during the winter than the summer and consequently may not take up very much TCAA during the winter months.

The pattern of TCAA concentrations with year class at the two sites is also very interesting. Figure 4.4 and 4.6 show a marked pattern of increasing TCAA with needle age at Dunslair Heights during both growing seasons. The pattern shows an accumulation of TCAA with increasing needle age as noted previously, which is observed in all the months sampled in 1998 and many in 1999. Conversely, at Venlaw (Figure 4.5 and 4.7) the same pattern is not displayed in any monthly samples except December 1998. If it is accepted that there was no accumulation of TCAA with age at Venlaw what is the explanation for the different patterns at each site?

One suggestion is that the needles of different year class have different metabolic activities which decrease with age, meaning a build up of TCAA in older needles. Another suggestion is that as the needles age their wax layer and stomata become increasingly damaged by pollution etc. and so the ability of the needles to control their water loss via their stomata is reduced (Cape & Percy 1996). This uncontrolled water loss would mean that more water would be drawn through the older needles via the transpiration stream, facilitating higher uptake of TCAA into the senescent needles. The combination of decreased metabolism and/ or increased uptake would explain the striking pattern found at Dunslair Heights. The reverse of this may explain the absence of such a pattern at Venlaw. The lack of high pollution
exposure, especially from cloud and fog, at the lower site may mean the trees generally, and older needles in particular, are less damaged than those of the same year class at Duns Lair Heights. This may mean needles at Venlaw are more able to control their water loss and may also have more effective metabolism processes. The warmer temperatures experienced at the lower site may also lead to faster metabolism of TCAA than at the more elevated site, which may continue for longer during the year. This would explain why all four year classes of needles at Venlaw generally contain similar TCAA concentrations. Exceptions to this pattern, such as the increasing profile found on 23/12/98, might be explained by a detailed examination of the meteorological conditions.

Comparison of the data obtained for the two sites shows that the concentrations of TCAA are generally higher at Duns Lair Heights than at Venlaw (Figure 4.8 and 4.9). The average concentration of TCAA in the 1998 needles over the whole period was 38 ng (g dwt)$^{-1}$ TCAA at Duns Lair Heights compared to 28 ng (g dwt)$^{-1}$ TCAA at Venlaw. The concentrations at Venlaw are higher than at Duns Lair Heights in only 2 out of the 19 samples analysed. At the start of this project this finding might have been taken as evidence that the concentration of TCAA was primarily related to wet deposition, because of the relationship between elevation of the sites and the atmospheric wet deposition. However, these findings demonstrate the complex nature of TCAA uptake, distribution and storage within Sitka spruce. The role of metabolism is not well understood and complicates the assumed simple relationship between uptake and concentration of TCAA. The variations in metabolism with temperature (related to both time of year and site) and needle year class also makes the estimation of a degradation rate of TCAA within Sitka spruce needles difficult, let alone between different tree species, countries or ecosystems. It is not until these processes are better understood that TCAA cycling in the environment can be adequately described.

Until now the major input of TCAA to trees was considered to be atmospheric wet and dry deposition, but now consideration must be given to the soil as a possible source of TCAA rather than simply as a reservoir of the TCAA from atmospheric
input. The soil processes that control the release of TCAA from soil into the soil solution and the role of soil in the interception and degradation of atmospheric TCAA prior to uptake must be elucidated. An additional factor, which is discussed in Chapter 5, is the spatial variation in soil TCAA concentrations and its effect on the needle TCAA concentrations within a site. It would be wrong to conclude that the occurrence of TCAA in needles is simply related to wet deposition as the results reported here suggest a complicated cycling of TCAA by both metabolic and uptake processes, which are not fully understood. A discussion of the routes of uptake of TCAA into trees can be found in Chapter 7.

4.6 Conclusions

From the results obtained in this study it is clear that trees should not be used as bioindicators or historical records for exposure to chlorinated solvents and TCAA. Bioaccumulation of TCAA has not been found to occur in Sitka spruce during the lifetime of the needles. TCAA is constantly taken up, but metabolism occurring within the needles prevents accumulation. Metabolism is a combination of two processes occurring in the needles of trees. These are primary photosynthesis, which converts carbon dioxide to fixed carbon, and respiration, which converts stored carbohydrates to energy for repair processes. As needles get older the respiration rates increase and primary photosynthesis probably decreases, mostly because of shading of the older needles by newer ones. The degradation of xenobiotics such as TCAA is likely to be linked to the capacity of the needles to perform primary photosynthesis rather than respiration. This is consistent with the data that has been presented in this study, as respiration is active even in older needles and during winter, so seasonal decreases in TCAA are more closely correlated with the changes in photosynthesis. If the rate of metabolism is greater than the uptake then the TCAA concentrations fall, whereas if the rate is slow compared to the TCAA uptake the concentrations increase. The complicating factor within trees is that the rate of metabolism is not constant either temporally or spatially and so this means that differences in concentrations cannot be related to historical input. Metabolism varies considerably with site and time of year as a direct effect of temperature and light levels. It appears that during winter the destruction of TCAA stops and levels of
TCAA increase. The lower uptake of water due to lower transpiration reduces the uptake of TCAA from soil, so the effect is not as marked as may have been expected. It is the magnitude and variability of the degradation rates that must be determined to fully understand the role of TCAA in a forest ecosystem.

This study of the two sites at Glentress Forest has not defined the route of uptake of TCAA by the trees. The finding of a relationship between increased wet deposition and TCAA concentrations between the sites does not prove a link between the two, due to the different growing conditions and metabolic rates of trees at Dunslair Heights and Venlaw. Research to elucidate the route of uptake by trees and a discussion of the probable routes of uptake in a forest ecosystem can be found in Chapter 7. Additional processes such as transport of TCAA from Year C needles to older needles (Matucha et al. 2000a & b) may also explain some of the fluctuations observed in younger needles. This may be another removal mechanism for TCAA as after translocation to older year classes, these may be dropped and TCAA expelled.

It must also be determined whether the whole amount of TCAA present in the trees is derived from atmospheric deposition or whether there is an additional contribution, perhaps from in situ production in soil. The role of the soil is either as a reservoir for atmospheric input or a reactor leading to the production and/ or degradation of TCAA, which subsequently releases TCAA to the trees via its roots. Results from a monitoring programme of TCAA in soil can be found in Chapter 5.

It can be concluded that the measurement of TCAA concentrations in trees cannot be related to historical trends of input of TCAA as a steady state concentration is likely to be maintained by tree processes. The trees appear to degrade TCAA taken up from their environment producing as yet undetected metabolites. The presence of TCAA at concentrations <200 ng g^-1 (dwt) does not appear to produce physiological effects detrimental to tree health at Glentress Forest, which is discernible to the eye.
Chapter 5

Trichloroacetic acid in soil

5.1 Introduction

Despite the increased levels of interest in trichloroacetic acid (TCAA) in the environment, particularly in forests, its source is still not well characterised. The atmospheric production of TCAA from its chlorinated precursors has been discussed in Section 1.1. The transfer of TCAA from the atmosphere to tree needles has been assumed to occur almost exclusively via wet deposition and subsequent uptake by plants through their roots, which is deemed the major uptake pathway (Schroll et al. 1994; Sutinen et al. 1995). However, the role the soil plays in uptake has not been well studied and it is assumed not to act as a simple conduit of TCAA from atmosphere to tree via the transpiration stream. It is still unclear, and a matter of debate, as to whether TCAA concentrations found in the environment can be fully accounted for solely by the anthropogenic source or whether another source must be sought (Juuti & Hoekstra 1998; Frank et al. 1999). If a greater understanding of the cycling of TCAA in an ecosystem is to be achieved then the origin, concentrations, behaviour and lifetime of TCAA in the soil environment must be defined experimentally alongside measurements of TCAA in other compartments. It must be determined whether the TCAA present in the needles of trees is related to TCAA in the atmosphere via soil uptake or whether it reflects the TCAA concentrations of the soil with only a minor atmospheric contribution.

It has been suggested that TCAA in soil occurs from several in situ production processes, both anthropogenic and natural, as well as wet deposition. One such anthropogenic production process is the formation of TCAA from the treatment of polluted groundwater with UV light. Hirvonen et al. (1996) showed that groundwater contaminated with tri- and tetrachloroethene could be decontaminated using UV light, which led to the production of TCAA. This process may well occur in the surface soil after atmospheric dry deposition of these chlorinated precursors,
but is not possible deeper in the soil as solar radiation cannot penetrate it. A study by Henschler (1977) has also shown that these chlorinated precursors may be converted to TCAA within microbiological organisms. Other sources of TCAA may be from various combustion processes. Hoekstra et al. (1999a) suggested that according to the trace chemistry of fire theory all types of oxidised chlorinated organic compounds could be formed in the presence of chlorine and suggests forest fires may be a significant source, as well as incineration of municipal waste. A small number of measurements from incinerators (Mowrer & Nordin 1987) has determined the concentration of TCAA in their flue gases ($2 \pm 1$ ng l$^{-1}$) to be 4 orders of magnitude higher than measured in urban air (Section 6.4.2.1). However, it was also suggested that TCAA may be formed in the soil from a totally natural production process.

The initiation step, which is important for the natural formation of TCAA in soil, is the formation of reactive chlorine species such as hypochlorous acid from chloride and hydrogen peroxide by a peroxidase-mediated reaction (Neidleman & Geigert 1986). Next the reactive chlorine species chlorinate the organic matter in a non-specific way. Peroxidases are ubiquitous in the environment, but it is only the chloroperoxidase enzymes (CPO) that produce reactive chlorine species. The activity of these enzymes depends on the species and pH but the optimum chlorination yields occur at pH 3-6 (Hoekstra et al. 1999b). This corresponds to the typical pH range of soil within a coniferous forest of 3-5 (Avery 1990).

Walter & Ballschmiter (1992) reported the CPO-mediated formation of chloroform from simple organic compounds and Asplund et al. (1993) were successful in isolating a soil extract with halogenation potential. Hoekstra et al. (1995b) demonstrated the formation of both TCAA and chloroform from humic acids. Hoekstra et al. (1998a) spiked the soil of a Douglas Fir forest with Na$^{37}$Cl and detected the production of CHCl$_3$ enriched with $^{37}$Cl. This evidence demonstrated that small organic compounds present in the soil, like acetic acid and citric acid could be chlorinated to compounds such as CHCl$_3$ and TCAA via some soil chlorination process. A great deal of work has been done to identify which enzyme(s) are
responsible for the specific chlorination reactions involved (Field et al. 1995; Hoekstra et al. 1998b) but without success.

From the study of hypochlorous acid in disinfection and bleaching processes CHCl₃ and TCAA were identified as the main chlorinated compounds of low molecular mass (Boyce & Hornig 1983). The reaction mechanism proceeded by the chlorination of the aromatic rings in the humic acid structures and not by the chlorination of aliphatic chains via the haloform reaction. Figure 5.1 shows a reaction scheme where TCAA and chloroform are formed from a resorcinolic structure which is a common structural element of humic material. The final ratio of CHCl₃ and TCAA depends on the structural elements of the humic material (Boyce & Hornig 1983). All di- and trihydroxy- substituted aromatic structures give rise to the formation of CHCl₃ and TCAA. Despite the large differences observed for individual substructures the overall result of chlorination of humic acid is pH dependent. If the pH is less than 7 then a higher yield of TCAA is obtained, but if pH is greater than 8 then the formation of CHCl₃ is favoured. With constant pH and Cl₂ dose, water samples containing humic materials produce a linear correlation between the concentration of TCAA and CHCl₃ (Reckhow et al. 1990).

Figure 5.1: The formation of CHCl₃ and TCAA from a resorcinolic structure (* means $^{13}$C)
Extrapolation of measurements in water samples led to the hypothesis that a positive correlation between TCAA and CHCl₃ would be found if a natural formation in the soil existed. However, a positive correlation could also be explained if TCAA were degraded to CHCl₃ by decarboxylation in the soil. Hoekstra et al. (1999b) argued that the amount of chemical degradation was negligible and the biodegradation processes in the soil did not produce CHCl₃, but instead CO₂ and chloride. This meant that a correlation between the TCAA concentration in soil and the concentration of any chlorinated solvent in soil air was evidence for a link in the formation process of TCAA. Hoekstra et al. (1999b) found a positive correlation (R² = 0.82) between CHCl₃ in soil air and TCAA in the soil in both their own results and the statistical analysis of those of Plümacher (1995) (R² = 0.97), but found only very weak correlations between TCAA and the concentration of other possible chlorinated precursors. It was concluded that the CHCl₃ in those soils was not produced from chlorinated solvent precursors nor decarboxylation, but instead was a novel indication for the natural formation of TCAA in soil.

The gap in knowledge in the soil compartment is the lack of widespread TCAA measurements at variety of sites, and where forest sites have been measured, the types of forest. TCAA concentrations were measured in Douglas Fir and Beech forests as well as peat bog and moor. Some of the recoveries achieved were poor (Hoekstra, Pers. comm.) and some are expressed from single soil samples (Hoekstra et al. 1999b). The measured TCAA concentrations were quite low with a range of 0.2 to 4.6 ng (g dwt)⁻¹ TCAA. Previously cited results by Plümacher (1995) and Frank (1988) showed higher concentrations with greater variability; 1.4 to 120 ng (g dwt)⁻¹ TCAA and 20 to 380 ng (g)⁻¹ TCAA respectively. The results quoted by Plümacher (1995) were expressed relative to dry weight and were determined in German pine forests using a HSGC technique. Frank (1988) sampled 20 cm soil cores but it is not clear from his paper whether the values were quoted relative to fresh or dry weight and whether the leaf litter had been removed before sampling.

The 3 studies of TCAA in soil (Frank 1988; Plümacher 1995; Hoekstra et al. 1999b) were at a range of sites and soils, but with generally a small number of samples.
The combination of the lack of clarity in presentation of results and the high standard deviations achieved make comparison between, and subsequent interpretation of, these results difficult. The high standard deviations may be due to analytical variability, but may also be explained by sample variability, as soil is known to be very heterogeneous. A more comprehensive study of TCAA in soil is required, by sampling soils from different types of site (forest/ moor/ heath) and as a time series to observe any seasonal variation. A common method of presentation of results (relative to fresh/ dry weight/ total organic matter) and the use of a standard method of sampling or analysis is also required to enable comparison of results in the literature and would enable the identification of patterns.

A further topic of research in the area of soils has been the effect of organic matter and humic material on the presence and lifetime of TCAA in soil. The half life of TCAA in soil is quoted as 14 to 90 days (Worthing & Hance 1991) and has been used as evidence for the fact that TCAA present today is not carried over from the application of the herbicide in the past. The processes that lead to the removal of TCAA have been studied but are still not well understood, but half life is likely to vary according to soil type, soil pH, temperature, moisture etc. The soil ecosystem has always been seen as a sink for the input of atmospheric TCAA, but it is still not certain whether the soil acts as a reservoir for TCAA or shows a steady state between accumulation, production and degradation. Volatilisation of TCAA from soil is unlikely due to its high Henry’s Law constant (Bowden et al. 1998) and low vapour pressure (Daubert & Danner 1989-1991). Photo-decomposition is also unlikely as the sun is unable to penetrate beyond the soil surface. The most likely decay process is microbiological degradation, which would occur by detoxification to CO₂ and chloride and not CHCl₃ (Kearney et al. 1969; Weightman et al. 1992; Yu & Welander 1995). The degradation rate has been observed to be faster in organic rich soils, which was explained by the higher population of micro-organisms (Barrons & Hummer 1951). Torstensson & Hammarström (1981) showed that the highest degradation rate occurred in the upper 0 to 5 cm soil layer due to the availability of nutrients and high organic matter content. It was estimated that the half life for
mineralisation of TCAA to chloride was between 10 and 68 days for organic carbon contents of 7.5 and 0.5%, respectively.

A much ignored process in soil is the possibility of adsorption or inactivation by binding. It was suggested that adsorption of TCAA on anionic-soil colloids (Kearney et al. 1965) and clay minerals (Goring 1967) was of minor importance due to its high water solubility but that there could be adsorption on iron and aluminium oxides. It is also assumed that the mobility and possibility of leaching of TCAA is high. These factors must be borne in mind when discussing the behaviour and results of soil experiments using TCAA.

Haiber et al. (1996) suggested that TCAA added to a lysimeter disappeared completely in the system. It was concluded that because there was no increase in CHCl₃, the TCAA was either microbiologically degraded or adsorbed to the soil. It was also determined in the same research that if TCAA was added to water containing humic acid at concentrations in the range of micrograms per litre, the recovery of TCAA dropped to 10% within 2 hours. This also suggested either very rapid breakdown or adsorption of TCAA by the humic acids. Haiber et al. (1996) concluded that the levels of TCAA present in bog waters containing high dissolved organic carbon should be low due to the same processes. However, this hypothesis was found to be untrue as bog water from Germany was found to contain 300 to 1000 ng l⁻¹ TCAA, which was explained by a possible natural formation occurring within the bog. It is likely with further work and a better understanding that these results could be explained or hypotheses confirmed.

The aim of the present study was to investigate possible temporal changes and seasonal effects on TCAA content of soils at Glentress Forest.

### 5.2 Sampling site

The site used for the analysis of soil for TCAA was the same as that sampled for needles, Glentress Forest (Section 4.2), in the Southern Uplands of Scotland. The two sampling areas vary significantly with Duns Blair Heights (elevation 602 m) being
a peaty, organic rich soil with Sitka spruce forest of 35 to 45 years old, whereas Venlaw (elevation 325 m) has a brown earth/ mineral soil and a younger planting (15-20 years old).

5.3 Sampling programme

Samples were taken at the Glentress Forest site over the period March 1999 to August 2000. Routine samples were taken during the four seasons at a variety of sampling spots at the two sampling sites. These samples were taken from the top 15 cm of soil using a trowel after any plant material or leaf litter was removed. Soil cores were also taken to investigate vertical variability. Two separate corers were used. Initially a corkscrew corer was used which bored into the ground and when removed contained soil from the different depths. The problems encountered with this technique include low amount of sample and crumbling of the core. This led to the use of a knife-edge corer. The corer was hammered into the soil to the required depth. The soil core was removed from the corer using a rubber-ended plunger. The soil cores were cut into sections in the field.

The samples were stored in a sealed sampling bag overnight at 4°C in a refrigerator. The following day the soil was sieved using a 2 mm mesh sieve and any stones, twigs or plant material was removed. The preparation of soil samples for HSGC analysis was done on the same day as sieving so as to minimise any degradation of TCAA.

5.4 Experimental

5.4.1 HSGC analysis

Soil was analysed by automatic HSGC as outlined in Chapter 3. From the well mixed sieved soil, 1.000 g (± 0.001 g) of the sample was transferred to glass headspace vials previously heated and cooled to drive off any CHCl₃. To these vials 1 ml of degassed HPLC water was added and the vials were crimped with PTFE coated rubber septa. Vials were prepared in quadruplicate to allow 3 replicate sample and 1 blank determinations. Unless the samples were to be analysed
immediately the sample vials were kept in a freezer at -10°C to stop any possible breakdown of analyte.

The procedure for HSGC analysis of TCAA in soil was the same as outlined for needles in Section 4.4.1. The partition ratio determined for 1g of soil was 1.98 as reported in Section 3.4.2.9 and was used in the same way as outlined in Section 3.3.

5.4.2 Loss on drying

The variation in the water content of different soils was large depending on the site, the position within a site and the sampling date. For example, the water content of Dunslair Heights soils in February 2000 varied between 55 and 74 % depending on location. This variation means that when weighing 1 g of sample there is contrasting amounts of soil material in the vial depending on the sample. The loss of mass on drying was used to express the TCAA concentrations in terms of sample dry weight.

The experimental procedure of loss on drying for soil was the same as for needles outlined in Section 4.4.2. The loss on drying procedure produced results that were within 1% for duplicate samples (n=40). This was probably caused by the sieving and mixing of the soil which made it as homogenous as possible. For this reason loss on drying of routine samples was performed on one sub-sample of the homogenised soil.

5.4.3 Loss on ignition

As suggested by Hoekstra et al. (1999b) the expression of results in relation to soil organic matter can be a useful parameter. The organic matter varies dramatically between the two sites and typically is 15 –20 % fwt. at Dunslair Heights and 9-12 % fwt. at Venlaw. The use of results in relation to soil organic matter enables comparison between samples that on a fresh or dry weight basis may seem to give very different results.

The organic matter content of a soil sample was determined by the loss on ignition measured on the oven-dried sample. The sample was weighed accurately into a
pyrex beaker and heated at 500°C for 8 hours in a furnace. After cooling the beaker was re-weighed and the weight loss determined. Loss on ignition has been performed at temperatures between 450 and 550°C as outlined by Nelson & Sommers (1982). It was found that the reproducibility of this method was good, with duplicate results always within 1% of each other (n=40). For this reason the loss on ignition for routine samples was performed on one sub-sample of the homogenised soil.

5.5 Results and discussion

5.5.1 Routine soil sampling at Glentress Forest

In order to present the large number of results in the most useful way the data are presented as separate graphs for each of the types of site within the sampling programme. For example, samples taken within a forest stand are presented together and are separate from samples taken from open moor. There is no difference between the sampling procedure or analysis, but this differentiation makes interpretation of results easier. The map in Figure 5.2 shows the locations from which samples were taken at Glentress Forest and the sample numbers which are referred to in the text. The soil samples have specific sample numbers e.g. DH1 with the sampling date in parentheses.

5.5.1.1 Soil from open moorland

The TCAA concentrations determined in soil sampled from open moor at Dunslair Heights are shown in Figure 5.3. The forest at Dunslair Heights is divided by a stretch of open ground acting as a boundary between two land owners. This land is covered with grass and heather and has been sampled regularly throughout the research (location 1 in Figure 5.2). It can be considered to be a control area for the effects of forest on soil processes as it receives similar input from precipitation as the tree stands but has no influence from forest conditions such as leaf litter or interception of cloud by trees. Any temporal differences solely due to atmospheric input should be apparent in these samples.
Figure 5.2: A map showing sampling locations at Dunsclair Heights
The results determined for the open ground show no seasonal changes from May 1999 through to April 2000. The concentrations of TCAA relative to soil fresh weight are in the range 5 to 10 ng (g fwt)$^{-1}$ TCAA and if corrected for water content are 15 to 45 ng (g dwt)$^{-1}$ TCAA. The peak in TCAA in September is not statistically different from the other data as this set of results has a high standard deviation. DH3 (29/9) sample taken at a different part of the moor confirms that this peak value is anomalous. The water content and total organic matter content remain constant, with the highest soil water content in September, which is the month with the second highest rainfall in 1999 (Figure 6.4). However, this does not lead to higher TCAA fresh weight values. This suggests that throughout the moor the soil is relatively homogenous, which may be because this area was not ploughed for tree planting. The constant TCAA concentrations throughout the year suggests that the balance between breakdown and production in this soil is constant despite the varying wet input throughout the year. The concentrations determined here are higher than in previous work which showed 2 ± 1 ng (g organic matter)$^{-1}$ TCAA for peat moor (Hoekstra et al. 1999b) and 8 ± 2 ng (g dwt)$^{-1}$ TCAA for agricultural land (Plümacher
1995), but this may be explained by some influence of being at a hill top site where there is likely to be greater input of TCAA from cloud and rain (Chapter 6).

5.5.1.2 Soil from forest rides

Figure 5.4 shows the results of TCAA determinations from soil samples taken on rides at Dunslair Heights. The rides are present mostly as firebreaks between tree stands and are approximately 6 metres wide. They are in close proximity to the trees but are covered with thick grass and heather. These samples were taken to identify if any TCAA maxima seen within tree stands were evident a short distance outside the stand.

![Figure 5.4; TCAA temporal variation in soil from rides at Dunslair Heights (1999–2000).](image)

This set of results is quite small and therefore it is hard to determine a temporal trend. The samples have been taken from only two rides. Samples DH1(29/9) and DH3(9/2) were taken from location 2 behind the radio station, whereas the other samples were all taken from location 3 to the south-west of the radio station. The main difference between the two sites is that location 2 has a lower organic matter content (12% fwt) than location 3 (15% fwt), which explains the larger correction when expressing TCAA relative to organic matter for samples at the first location. This is demonstrated for the two samples from 29/9/99 (Figure 5.4) where a similar
concentration, approximately 30ng (g dwt)$^{-1}$ TCAA is measured, but it becomes significantly larger for DH1 after expression relative to soil organic matter. The TCAA concentrations are generally larger, but in the same range as the samples from the open moor. This may have been expected as there is no direct input from the trees at these sites.

5.5.1.3 Soil from Venlaw tree stands

Figure 5.5 shows the results obtained from soil sampled at Venlaw. Samples were taken along the side of the road opposite the rain sampler at Venlaw. All of the samples shown in Figure 5.5 were taken from within the stand of trees from below the road. Due to the young age of the stand (15-20 years) the ploughed furrows are still very clear. The trees are also very well spaced (2 m) and as yet no leaf litter layer has accumulated. This soil here is a brown, mineral soil and is particularly heterogeneous with many stones. The soil is very well drained and typically has a water content of 20 to 40% and a total organic matter content of 8 to 13% (fwt) which is much lower than at Dunslair Heights.

![Figure 5.5; TCAA temporal variation in soil from Venlaw forest (1999-2000)](image)

*Error bars are standard deviations of triplicate analyses*
Considering the soil characteristics it is no surprise that the TCAA concentrations are lower here [5 to 35 ng (g dwt)^{-1} TCAA] than those for open ground at Dunsclair Heights [15 to 45 ng (g dwt)^{-1} TCAA]. This could be explained by two factors. The poor retention of water means that any leaching of TCAA down the soil profile will be greater and the soil would not therefore act as a reservoir for TCAA. Additionally the lower organic matter content of the soil would suggest that, if natural formation does occur, it would be higher at Dunsclair Heights than Venlaw. If TCAA is expressed in relation to soil organic matter there is a huge correction due to the smaller organic content of the soil. The concentrations expressed in this way are in the range of 20 to 180 ng (g organic matter)^{-1} TCAA. This is far greater than determined in any of the other non-forested sites discussed previously and shows the presence of TCAA despite low organic matter content and poor water retention.

The heterogeneity of the Venlaw site is well demonstrated by the 2 soil samples from 29/9/99. Sample V1 was taken from the crest of a furrow whereas sample V2 was taken at the base of the same furrow. It is clear that the amounts of TCAA found at the top of the furrow were greater than at the bottom. It is not clear why this should be, as the water and organic matter content were very similar for both samples and perhaps demonstrates the heterogeneity present in the soil at this site.

Samples V1 and V2 (9/2/00) were taken on the edge and in the centre of the stand respectively to investigate whether edge effects are important. The results in Figure 5.5 showed evidence of greater TCAA in soil from the centre of the tree stand. The trees at Venlaw do not intercept cloud water as there are very few cloud events here and so edge effects are not likely to be observed. Similarly on 12/4/00 one sample was taken at the base of a tree (V1) and one further away to look for possible effects of stem flow on soil TCAA concentrations. Concentrations of ions in stemflow can be up to 10 times greater than in rain (Cape et al. 1989). However, the soil samples showed no significant difference. A more detailed study would be required to investigate the potential contribution of stemflow.
The heterogeneity that has been demonstrated at Venlaw makes quantitative measurements in the soil difficult. However, the duplicate samples taken on 1/12/99 and 12/1/00 from the same site and sample type show remarkably good agreement at each sampling date. It is not clear with this level of sampling whether the differences between these dates represents an increase in soil TCAA, or is simply a reflection of spatial heterogeneity.

5.5.1.4 Soil from tree stands at Dunslair Heights

Figure 5.6 shows the TCAA concentrations measured from the soil beneath different tree stands at Dunslair Heights between May 1999 and August 2000. The results from Venlaw (Figure 5.5) are relatively homogenous compared with measurements from Dunslair Heights (Figure 5.6). Three results are greater than 200 ng (g fwt)$^{-1}$ TCAA and one is greater than 350 ng (g fwt)$^{-1}$ TCAA. This suggests that the processes that lead to such high concentrations are occurring only in the soil beneath the trees at Dunslair Heights. These extremely high results were only found in soils in a tree stand, but not every soil from a tree stand contains very high TCAA concentrations. However, finding such large concentrations in different parts of the forest on 5 different dates, with good agreement of the replicate analyses, suggests that the values are real. The results expressed in terms of dry weight, of up to 700 ng (g dwt)$^{-1}$ are far higher than any previously cited in the literature by Frank (1988) of 20-380 ng (g dwt)$^{-1}$ TCAA or by Plümacher (1995) of 1.4-120 ng (g dwt)$^{-1}$ TCAA.

The temporal trend at Dunslair Heights in the smaller data is more clearly seen by plotting the data on a smaller vertical scale (Figure 5.7). The results of the initial sampling during May and June 1999 from the tree stands at Dunslair Heights show that TCAA was detected in the range 20 to 45 ng (g dwt)$^{-1}$ TCAA. These results relative to soil dry weight are higher than were detected at other locations at Dunslair Heights in the same time period, but the difference is not statistically significant. If the concentration relative to total organic matter is considered, it is clear that initial results from the stands at Dunslair Heights are generally lower than those from stands at Venlaw. If TCAA is formed from organic matter in the soil, higher concentrations may have been expected at Dunslair Heights than Venlaw. Another
Figure 5.6; TCAA temporal variation in soil from tree stands at Dunslair Heights (1999-2000)

(Error bars are standard deviations of triplicate analyses)

Figure 5.7; TCAA temporal variation in soil from tree stands at Dunslair Heights (1999-2000) [0-250 ng g\(^{-1}\) scale]

(Error bars are standard deviations of triplicate analyses)
factor to consider is that the initial samples were taken in the summer. This was when the temperature was highest and one might expect greatest production by the soil, but microbiological degradation processes may be favoured leading to increased breakdown of xenobiotics such as TCAA. The input of TCAA from rainfall between June and August 1999 were close to the average for 1999 (see Chapter 6), so low TCAA concentrations during summer cannot be ascribed to lower accumulation from wet deposition. Measurements of soil TCAA during August 2000 display some characteristically high soil concentrations, which demonstrates that there is no seasonal variation in TCAA formation/degradation in soil.

The results determined in the samples taken in the winter period show the previously mentioned high TCAA concentrations. It is worth noting that the samples DH2 and DH4 (1/12/99), which were both situated under a tree stand (location 4 and 5) display concentrations of 230 and 7 ng (g fwt)\(^{-1}\) TCAA respectively. It is clear that some process or factor is required to account for this heterogeneity, and that this factor is not uniform between different stands. The sampling of 12/1/00 was designed to test whether the high TCAA found on 1/12/99 was a one-off finding or if these high concentrations were common to this stand. DH2 and DH3 were taken 100 metres apart at a distance of 5 metres into the stand (location 6 & 7) and a further sample (DH4) was taken further into the stand (location 8). This was designed to elucidate any edge effects, which were present due to, for instance, interception of cloudwater by the trees. DH2 does show a much higher TCAA concentration [81 ng (g fwt)\(^{-1}\)] than DH4 [21 ng (g fwt)\(^{-1}\)] which suggests the existence of edge effects. However, the concentration in the other edge sample (DH3) was much lower than the other 2 samples with only 8 ng (g fwt)\(^{-1}\) TCAA. The only conclusion possible is that the heterogeneity present within a stand is huge. For an estimation of this heterogeneity see Section 5.5.2.

Samples from 9/2/00 were taken from similar sites as on 1/12/99 (location 5 & 6). It is not surprising that DH2 (location 6) produced similarly large TCAA concentrations, but it is apparent that the TCAA at DH1 (location 5) increased significantly over the period from 8 to 17 ng (g fwt)\(^{-1}\) TCAA. This may demonstrate
an accumulation over time, but is more likely to be caused by soil heterogeneity and spatial variation. The sample set from 12/4/00 is also very interesting. The soils in stand A that normally showed high concentrations are modest, with 8 ng (g fwt)$^{-1}$ TCAA at both 10 metres into the stand (DH2; location 9) and in the centre (DH3; location 10). However, the largest concentration so far was measured in stand B, behind the mast (DH4; location 11) with 390 ng (g fwt)$^{-1}$ TCAA. This is very significant, as up to this point only one stand had shown these enormous concentrations, which may have been due to specific geological or meteorological conditions such as wind direction. However, the evidence of sample DH4 (12/4/00) is that these large concentrations exist throughout the site. It is not yet clear what properties or conditions lead to these levels but if they exist throughout this site then high TCAA should also be present. One idea for the cause of the high TCAA may be the existence of drip points throughout the tree stands. These areas are a focus of throughfall from the trees and may concentrate not only TCAA directly from rain and cloud, but may also scavenge TCAA from the surface of the needles with which it is in contact. If this is true it is not clear why a similar effect is not seen along the whole stand edge where greater cloud interception by trees occurs when trees have the same prevailing wind direction (c.f. location 6 & 7).

The high TCAA concentrations have been shown to exist in the winter period and not the summer, but it is not certain whether this was due to chance with such a small sample number or due to actual environmental conditions. To test this a further 5 samples were taken in tree stands during August 2000. DH1, DH3 and DH4 were sampled in stand A at location 6, 12 & 13 respectively. DH5 and DH6 were sampled in stand B behind the radio station (location 14 & 15), 10 metres from the stand edge and 3 m apart. The aim of this sampling was to demonstrate whether high concentrations of TCAA were detectable during summer. Samples DH1 and DH3 had low concentrations [8 & 6 ng (g fwt)$^{-1}$ TCAA], but DH4 had a higher concentration [90 ng (g fwt)$^{-1}$ TCAA]. DH4 was taken under a thick tree canopy so could not be explained by direct wet input and again suggests that there is no edge effect, but large heterogeneity. DH6 had a lower concentration [20 ng (g fwt)$^{-1}$ TCAA], but in the nearby sample DH5 there was 270 ng (g fwt)$^{-1}$ TCAA. This again
shows the presence of large TCAA concentrations in different tree stands and the variation over short distances. The results from August 2000 prove that no seasonal variation is observed as high concentrations can be measured in tree stands throughout the year.

To estimate whether the concentrations found in this study can be explained purely by accumulation of atmospheric input a 'back of the envelope' calculation has been attempted. The atmospheric input of TCAA to forest at Dunslair Heights from April 1999 to April 2000 was 900 \( \mu g \) m\(^{-2}\) by rain and 125 \( \mu g \) m\(^{-2}\) by cloud (Chapter 6). This is equivalent to 100 ng cm\(^{-2}\). If this enters soil to a depth of 10 cm then the soil concentration should be 10 ng TCAA cm\(^{-3}\). For soil density of 1g (fwt.) cm\(^{-3}\) (Table 8.4), the concentration of TCAA present in soil should be approximately 10 ng (g fwt\(^{-1}\)) assuming only atmospheric input. If the high results are ignored the concentration of TCAA in soil under tree stands is 11 ± 5 ng (g fwt\(^{-1}\)) TCAA, which is in good agreement with the calculated value. However, it is much lower than the peak values determined of >300 ng (g fwt\(^{-1}\)) TCAA, and suggests that either there is another source of TCAA, or that the soil is able to accumulate TCAA over time, or that the wet deposition to the forest floor is not at all uniform.

One possible source of TCAA to the soil is the leaf litter. At Dunslair Heights there is a particularly large accumulation of dead and decaying needles present under all the densely packed tree stands that have been sampled. It is known that TCAA is present in living needles at concentrations of up to 150 ng (g dwt\(^{-1}\)) TCAA (Chapter 4) and so if some of this TCAA is leached to the soil it could be a significant source. To investigate the magnitude of TCAA present in this compartment samples of this leaf litter were taken in the tree stand at location 6 on 12/1/00, location 9 on 14/8/00 and location 7 on 1/11/00. It was analysed by HSGC using the same procedure as for needles (see Section 4.4.4). The results are shown in Table 5.1.
<table>
<thead>
<tr>
<th>Sampling date</th>
<th>TCAA ng (g fresh weight)</th>
<th>TCAA ng (g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/1/00</td>
<td>35.7 ± 2.0</td>
<td>170.1 ± 9.4</td>
</tr>
<tr>
<td>14/8/00</td>
<td>33.1 ± 1.8</td>
<td>141.5 ± 7.8</td>
</tr>
<tr>
<td>1/11/00</td>
<td>26.0 ± 2.7</td>
<td>166.4 ± 9.2</td>
</tr>
</tbody>
</table>

Table 5.1; TCAA concentrations in leaf litter at Dunslair Heights

(Error ranges are standard deviations of triplicate analyses)

If only 10 ng (g fwt) TCAA in soil is explained by atmospheric input then the TCAA present in leaf litter is very significant. It is possible that precipitation leaches TCAA from the decaying needles as it permeates through the leaf litter to the soil. This would however suggest that TCAA should be found primarily in the soil surface and will decrease with depth, unless the mobility of the trichloroacetate ion is high. The concentration of TCAA in leaf litter is relatively constant from January to November at approximately 30 ng (g fwt) TCAA. This may be a continuous source of TCAA to the soil as the litter is continually added to and is not quickly degraded at Dunslair Heights.

5.5.2 Horizontal variability of TCAA in forests

Many of the results found during the routine sampling of soil at Dunslair showed that there was large spatial heterogeneity in the soil beneath tree stands. To investigate the variation in TCAA across a stand of trees, samples were taken along a transect in stand A at Dunslair Heights on 8/1/00 (Figure 5.1) at locations shown in Figure 5.8. The hypothesis was that differences between samples could be explained by their location within the forest, rather than simply due to random variations in soil. This was also an opportunity to discover whether the huge TCAA level determined in December 1999 was representative of the tree stand as a whole. Sampling started at location A (DH1) and moved across the stand to location B (DH5) and back into the stand at location C (DH8) with samples taken approximately every 10 metres.

The results (Figure 5.9) show that there was significant variability in soil TCAA concentrations across the stand, from 10 to 185 ng (g fwt) TCAA. The higher
Figure 5.8; A map showing the sample locations in stand A for the soil transect samples taken on 8/1/2000
results were detected in the soils sampled at the edge of the stand. Despite the sample nearest the edge (DH1) having a lower concentration than sample DH2 the higher results are possibly due to the interception of cloud and rain by the edge trees. Several authors have reported the edge effect at between 20 and 30 metres into a stand (Lindberg & Owens 1993; Weathers et al. 1995), which would support these findings. DH5 soil is also a sample from the stand edge, but does not have high concentrations, possibly because it is not in the direction of the prevailing wind. Samples DH3 and DH6 also show significantly higher TCAA than in most samples during the earlier routine sampling, with 38 and 31 ng (g fwt)$^{-1}$ TCAA respectively. The remaining samples have concentrations in the range 7 to 16 ng (g fwt)$^{-1}$ TCAA, which is similar to samples taken in open moor. The range of TCAA levels found suggests that the origin of TCAA production/accumulation is not uniformly spread within a stand, which is also supported by the concentration differences in samples from 12/1/2000 (Figure 5.7). The existence of drip points within a stand is a possible explanation, but would they be able to concentrate TCAA enough to produce such values? A full analysis of the soil properties using procedures such as sequential extraction might provide further useful information. An investigation into the binding properties of soil can be found in Section 5.5.4.

![Graph showing horizontal variation of TCAA concentration in forest soils](image_url)

**Figure 5.9; Horizontal variation of TCAA concentration in forest soils**

*(Error bars are standard deviations of triplicate analyses. N.B. sample DH2 analysed in duplicate only)*
5.5.3 Vertical variability of TCAA in forest soils

A horizontal variation of TCAA concentration in soils within a stand has been demonstrated and so the vertical variability of the soils with high TCAA concentrations was investigated by taking soil cores. It was expected that the maximum TCAA concentrations would be at the surface of the soil as found in German pine forest soils (Plümacher 1995). In that study the concentrations in the O horizon were 9-120 ng (g dwt)^{-1} TCAA compared to 2.4 -14 ng (g dwt)^{-1} TCAA in the A horizon, suggesting up to a 9-fold difference at the soil surface.

On 12/1/2000 at the locations where the routine samples DH2, DH3 and DH4 were taken a soil core of 30 cm depth was sampled as close to the routine samples as possible. The cores, which included the organic matter at the surface, were sectioned into 4 x 7.5 cm length samples. These were sieved and prepared as for the other soil samples. The results are shown in Table 5.2 expressed in terms of dry weight (dw) and organic matter (om).

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Core 1 (=DH2)</th>
<th>Core 3 (=DH3)</th>
<th>Core 5 (=DH4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCAA ng g^{-1} dw</td>
<td>TCAA ng g^{-1} om</td>
<td>TCAA ng g^{-1} dw</td>
</tr>
<tr>
<td>0- 7.5</td>
<td>1300 ± 100</td>
<td>1400 ± 100</td>
<td>63 ± 7</td>
</tr>
<tr>
<td>7.5- 15</td>
<td>1200 ± 20</td>
<td>5000 ± 100</td>
<td>65 ± 12</td>
</tr>
<tr>
<td>15- 22.5</td>
<td>3700 ± 180</td>
<td>10000 ± 500</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>22.5- 30</td>
<td>170 ± 10</td>
<td>340 ± 20</td>
<td>31 ± 2</td>
</tr>
</tbody>
</table>

Table 5.2; Vertical profile in soil TCAA concentrations in cores sampled at Dunslair Heights on 12/1/2000

(Results are mean ± standard deviation on triplicate analyses;
Cores referenced to nearby routine soil sample - shown in parentheses)

The individual sections were very different in both water content and organic matter content. For this reason the results have been expressed relative to dry weight and
organic matter. An example of this variability is that the organic matter content for the sections of core 1 were 20, 16, 3 and 32% fwt from the top to the bottom.

The results from the core samples are generally higher than the routine samples taken at the same date from within 50 cm of the core. The highest concentration in both the cores and routine samples were for core 1 (DH2). The concentrations in the sections of core 1 were in some cases an order of magnitude higher than the matching routine sample (Table 5.3). Core 3 and core 5 with between 20 and 70 ng (g dwt)\(^{-1}\) TCAA are in the same concentration range as determined in the associated soils (20 to 90 ng (g dwt)\(^{-1}\) TCAA). It is apparent that there is large scale variation within 10 cm depth.

<table>
<thead>
<tr>
<th></th>
<th>Core 1</th>
<th>DH2</th>
<th>Core 3</th>
<th>DH3</th>
<th>Core 5</th>
<th>DH4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng (g dwt)(^{-1})</td>
<td>1250</td>
<td>210</td>
<td>64</td>
<td>20</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>ng (g OM)(^{-1})</td>
<td>3200</td>
<td>530</td>
<td>80</td>
<td>50</td>
<td>100</td>
<td>120</td>
</tr>
</tbody>
</table>

Table 5.3; Comparison between the mean TCAA concentrations in soil cores (0–15 cm) and nearby routine sample

The most important pattern shown in Table 5.2 is that the high TCAA concentrations measured in core 1 do not decrease with depth until the last section of the core. In fact when considering the OM corrected results there appears to be a peak value in the third section. This value of 10 μg (g organic matter)\(^{-1}\) TCAA is far higher than anything previously reported in the literature. On closer inspection of this core one of the factors responsible for this high concentration is the water content. In the 15 – 22.5 cm section of core 1 the concentration is 250 ng (g fwt)\(^{-1}\) TCAA. This in itself is a high value but not extreme. However, the water content of this sample was 93%. Though this was measured in the same way as for other samples it has to be suspected whether this is an anomalous result.

Core 3 also showed no decrease in TCAA with depth, with a consistent concentration of 70 to 90 ng (g organic matter)\(^{-1}\) TCAA. Core 5 also displayed a large decrease in TCAA in the deepest section, similar to that found in core 1. This may be explained by the mobility of TCAA, which is influenced by the soil humidity (Gaber et al.)
1974). At 33% humidity TCAA was found to penetrate 15 cm in one day, whereas at a humidity of 5% TCAA remained in the upper soil layer for 25 days. Rainfall might also cause further transport to lower depths. Without knowing these parameters for each soil core it cannot be estimated whether the concentrations at depth are due to leaching of TCAA downwards or natural production.

It is also possible that TCAA could be bound to the humic material present in the soil (Haiber *et al.* 1996) with differences in organic matter functional groups at different depths accounting for greater binding and thus higher concentrations. Haiber *et al.* (1996) stated that in a study with 30 cm soil and peat lysimeters the TCAA which was introduced *via* rain disappeared to an undetectable level in water draining from the system. This was explained by either decomposition or adsorption with the prior option favoured. No trend of decomposition with depth was seen in the cores in Table 5.2, which suggested that the TCAA measured was actually bound to the soil and thus could not be leached. This hypothesis can only be tested by further work on the soil dynamics, but simple leaching experiments have been performed and are discussed in Section 5.5.4. A more detailed discussion could be achieved through the lysimeter experiments, if not only the aqueous leachates, but also the profile of soil TCAA concentrations with depth were determined. This would allow the direct measurement of changes in soil TCAA concentrations after its removal from the influent stream, to estimate if binding or decomposition was responsible for the decreased concentrations measured in the leachates.

Another consideration for soil TCAA is the concentration of TCAA in the organic matter layer consisting of decaying leaf litter. The concentrations measured in 3 samples (Table 5.1) had average concentrations of 32 ng (g fwt)⁻¹ TCAA. Therefore this could be a source of some of the TCAA present in the top layers of the cores, but would be more significant to the concentrations found in cores 3 and 5 than to the higher concentrations in core 1. However, if this source of TCAA is constantly replenished by more dropped needles it is possibly important in the cycling of TCAA from wet input to needles to leaf litter to soil and may explain some very high concentrations measured under tree stands.
5.5.4 Extraction of TCAA from soil

5.5.4.1 Results and discussion

The HSGC technique used in this work gives some advantages to determining the TCAA present in the soil. The ability to determine TCAA directly from soil rather than after an extraction procedure is a major advantage compared to the derivatisation techniques. It is assumed that all TCAA, whether non-bound in the soil solution or bound to the soil matrix, undergoes decarboxylation to Cl⁻ on heating. An investigation into the location and binding of TCAA in the soil was examined by a series of extractions using various reagents to leach TCAA from the soil. The effectiveness of extraction was tested by measuring the concentrations of TCAA in the soil extracts and the soil residue after extraction.

The first approach taken was to extract soil with HPLC-grade water, which was assumed would extract any non-bound TCAA present. The second approach was to use 1M nitric acid as an extractant. If the trichloroacetate was bound to anion exchange sites then it was thought that the strong acid would displace the TCAA, as trichloroacetate (TCA⁻) would be converted to its acid form below its pKa and hence disrupt any ionic binding. Also high concentrations of nitrate ions might displace TCA⁻, with low pH favouring desorption. The added advantage of this experimental methodology is that both the extracted solution and the soil after extraction can be reanalysed for TCAA to estimate recovery and % TCAA still bound. A further advantage was that a soil with a high TCAA concentration was discovered (DH2 9/2/00) and by basing experiments on this soil any blank TCAA contribution from HPLC-grade water and HNO₃ is considerably less significant. The results of the extraction experiments are shown in Figure 5.10 & 5.11 for extraction times of 2 hours and 16 hours respectively.

The results in Figure 5.10 show the initial extraction efficiency of TCAA from soil after 2 hours. Soil (10 g) was weighed into centrifuge tubes and 10 ml of either 1M nitric acid solution or HPLC-grade water was added. These tubes were sealed and shaken for 2 hours using a mechanical shaker. The tubes were then centrifuged and the supernatant solution removed. The tubes were centrifuged a second time to
remove any remaining solution. The supernatant was also centrifuged to remove any solid material. The solution was neutralised with NaOH solution and analysed by HSGC. The concentration of TCAA was determined in both HPLC-grade water and NaOH neutralised HNO$_3$ solution and used as a blank. The soil water extracts were not neutralised. To test whether TCAA remained bound to the soil, two soils extracted using water were reanalysed using the normal procedure to test for a complete mass balance.

![Figure 5.10; TCAA soil extraction experiment (2 hours)](image)

(Each bar represents the analysis in triplicate of a sub-sample of soil, an extract of a sub-sample of soil, or the residual soil after extraction. Initial soil was analysed prior to experiment. Extractions were performed on quadruplicate sub-samples of soil. Error bars represent standard deviation on triplicate analyses)

The extraction procedure (Figure 5.10) has not efficiently removed TCAA from soil with either water or acid. Seven of the eight extractions gave recoveries ranging from 0 to 6%, one of the water extractions gave a recovery of 18%. The water extracts appear to be more effective than with acid, but still give poor extraction efficiency. The results from the analysis of two of the soil residues remaining after water extraction proved that the TCAA was still present in the soil, possibly bound to the humic material or iron/aluminium oxides (Goring 1967). The extraction
procedure showed an excellent mass balance for TCAA of 100 ± 0.4% for the water extracted samples analysed, with no losses caused by degradation.

Due to the large variability within each set of 4 soil extracts, particularly with water, the experiment was repeated with an extended shaking time of 16 hours (Figure 5.11), as used by Hoekstra et al. (1999b) for analysis of TCAA in soils. The extractions with HNO₃ and water were performed on duplicate sub-samples of the same soil sample (DH2 9/2/00) as previously used. The soil extracts and the residues of two water-extracted soils were analysed by HSGC.

![Figure 5.11; TCAA soil extraction experiments (16 hours)](image)

(Each bar represents the analysis in triplicate of a sub-sample of soil, an extract of a sub-sample of soil, or the residual soil after extraction. Initial soil was analysed prior to experiment. Extractions were performed on duplicate sub-samples of soil. Error bars represent standard deviation of triplicate analyses)

The increased shaking time has not significantly improved the extraction efficiency (Figure 5.11). The reanalysed soil residues showed that 85% of the initial TCAA concentration remains bound to the soil. The reproducibility both between duplicate extractions with acid and with water and within the duplicates is extremely good. This suggests that the maximum recovery has been achieved using water and acid
with this protocol. The average recovery is 6% which shows that either this is the maximum TCAA that can be extracted or that only 6% of the TCAA in this soil is unbound. These results have implications for both the analytical methodology used to determine concentrations of TCAA in the soil and for previous assumptions of the behaviour of TCAA in soil.

A final extraction experiment was performed at acidic, neutral and basic pHs. A procedure by Scott et al. (2000) used a pH 10 extraction to remove TCAA from the soil matrix before analysis by derivatisation and GC-MS (Scott & Alaee 1998), which produced higher concentrations than determined using a water extraction and the same analysis. This suggested that basic solutions were more efficient at extracting TCAA from soil.

In this experiment extractions were performed on 3 sub-samples of soil (10 g) for each extractant. The extractants used were 0.06 M H₂SO₄ (pH 1), HPLC-grade water (pH 7) and 0.1 M NaOH (pH 13). In this experiment H₂SO₄ was used instead of HNO₃ as its low vapour pressure stopped any transfer of acidity to the analytical column which would cause damage to the stationary phase. The samples were shaken for 16 hours to ensure maximum extraction. The 9 extracts and 9 soil residues were analysed by HSGC to determine the extraction efficiencies at each pH (Figure 5.12).

A visual examination of the extracts revealed that the colour of the solutions changed from a very pale yellow colour at pH 1, to a light brown clear solution with water and a strong orange/brown opaque solution at pH 13. This strong colour was explained by the extraction of humic acids with the basic extractant. The TCAA concentrations measured in the extracts varied with the pH used, with the basic extracts having the highest concentrations and the acidic extracts the lowest, the reason for which is not known. One suggestion is that TCAA was associated with humic acids and as more were extracted in the basic extracts the concentration of TCAA increased. To test this hypothesis the humic acid precipitate could be removed by filtration and then either the precipitate analysed for TCAA directly or a
decrease in the TCAA concentration in the filtered solution after reanalysis could be investigated.

![Figure 5.12; Soil extraction experiments with acidic, neutral and basic conditions](image)

(Each bar represents the analysis in triplicate of a sub-sample of soil, an extract of a sub-sample of soil, or the residual soil after extraction. Initial soil was analysed prior to experiment. Extractions were performed on triplicate sub-samples of soil. Error bars represent standard deviation of triplicate analyses)

The amount of TCAA extracted is very low with 3, 6 and 10% for acid, neutral and base conditions respectively. The 6% recovery achieved in this experiment is in good agreement with the recovery achieved in the previous extraction experiment using the same conditions. The base extractions result in a 66% increase in TCAA concentrations compared to neutral extracts, which would explain why Scott et al. (2000) have observed higher concentrations using basic extraction than other authors using water extraction.

Another important aspect of the results is the TCAA remaining in the soil residues. The concentrations in the residues are highest after extraction with acid and lowest after basic extraction, which is consistent with the opposite trend in the extracts.
This shows that NaOH is a more efficient extractant than water and acid, but still approximately 90% of the original TCAA remains bound to the soil matrix. An important point is that there is no overall mass balance of TCAA in the experiment with 80% for acid and only 54% for the basic extractions. This is perhaps caused by losses due to degradation, as it has been reported that both TCAA (Worthing & Hance 1991) and CHCl₃ (Køppen et al. 1988) are unstable in alkaline conditions. This would mean that either TCAA is broken down during the extraction procedure leading to lower total TCAA concentrations, or that after decarboxylation CHCl₃ is degraded and therefore TCAA is underestimated by HSGC. An improvement to this experiment would be to repeat the protocol using an extractant at pH 8 at which TCAA is stable (Køppen et al. 1988). If losses have been experienced at pH 13 then the efficiency of basic extractions should be higher, which would mean NaOH was even more effective than the other extractants. Further work is required to prove this.

5.5.4.2 Implications of extraction experiments

First, these results create a problem analytically. The inability of the derivatisation protocol (Hoekstra et al. 1999b) to efficiently extract TCAA from soil means that previously cited results using this method are open to question. If the extractions are only 10% efficient then should the previously reported concentrations actually be 10 times higher? The spiking and recovery of TCAA from soils are notoriously unreliable (Hoekstra Pers. comm.). If recovery is to be determined by spiking soil with ¹³C-TCAA, does this recovery depend on the matrix to which the spike is added and how long it is left to equilibrate? Haiber et al. (1996) showed that if water containing humic acid was spiked with TCAA, its recovery after 2 hours was less than 10%. It was concluded that either adsorption or decomposition was the cause. The results in Section 5.5.4.1 imply adsorption or binding is the process occurring. Results reported using HSGC (Plümacher 1995) showed TCAA concentrations of 40 ng (g dwt)⁻¹ TCAA in pine forest soil compared to 0.2 to 0.3 ng (g dwt)⁻¹ TCAA in Douglas fir forest soil using the derivatisation method (Hoekstra et al. 1999b). This is obviously a complicated comparison as the soils are not necessarily the same and the differences may be authentic rather than analytical, but are the concentrations
really two orders of magnitude different? The distinct advantage of the HSGC method over the derivatisation method is that no extraction is required and direct soil measurements can be made, allowing the reanalysis of extracted soil residues after extraction. Could the difficulties faced by many researchers in the analysis of soil for TCAA be removed by the use of HSGC method?

Are the implications of these findings actually important? Is the determination of total TCAA in the soil a useful parameter? If the TCAA determined in this research is bound very tightly within the soil what are its environmental consequences? If the methodology used here, namely 16 hours of extraction, is ineffective at releasing TCAA is it possible that this TCAA is irreversibly bound? If this is the case, then the implication in a forest environment is that the TCAA will not be available in the soil solution and therefore would not be available for uptake via the tree roots. This may explain why 10-fold differences in soil TCAA concentrations at Dunslair Heights have not led to large variations in the needle TCAA concentrations. The implication is that the most important parameter to determine is the extractable/available TCAA, which as discussed above is well determined by the derivatisation method. Until the soil processes are defined these questions cannot be answered, but simple experiments such as reversible addition/extraction experiments would attempt to describe the dynamic and binding processes occurring. Simple measurements of TCAA in soil solution from lysimeters by HSGC would confirm what the extraction protocol by Hoekstra et al. (1999b) determines. In conclusion the most important piece of research would be to do a mass balance on a soil environment which would answer the question of binding and availability of TCAA and also the wider question of whether accumulation or production is occurring.

5.5.4.3 Limitations of HSGC for soil analysis
A possible criticism of the HSGC technique is that the CHCl₃ detected may not be produced from the decarboxylation of TCAA, but from some other source. A blank determination of background CHCl₃ may account for some sources, but there are two possible explanations for CHCl₃ in soil other than from TCAA. It was suggested that certain biotic and abiotic processes may produce CHCl₃ on heating (Schöler Pers.
It was suggested that this formation occurred at temperatures above 90°C and so during decarboxylation this extra contribution was produced and not corrected for. Another explanation was that trichloroacetaldehyde (chloral) would be decarboxylated to CHCl₃ on heating so increasing the apparent TCAA concentration. It was thought that chloral, if present, was unstable at 60°C and so would be determined in the blank vials (Frank et al. 1991). Two experiments were designed to test these hypotheses.

Previous work by Plümacher & Renner (1993) used decarboxylation conditions of 65°C for 72 hours to determine TCAA by HSGC. This was proved to be effective, but was thought too time-consuming for this research. If 90°C was a trigger temperature for CHCl₃ production, a comparison between protocols using the standard procedure (100°C for 90 minutes) and that of Plümacher & Renner (1993) (65°C for 72 hours) would prove it. The comparison was performed using a soil sampled at Dunslair Heights (14/8/00) which had a large TCAA concentration. The determination of background CHCl₃ in both cases was determined after soils were heated at 65°C for 90 minutes. The results for TCAA concentrations determined after background CHCl₃ correction are shown in Table 5.4.

<table>
<thead>
<tr>
<th>Decarboxylation temperature (°C)</th>
<th>65°C</th>
<th>100°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng (g fwt)⁻¹ TCAA</td>
<td>337 ± 28</td>
<td>267 ± 5</td>
</tr>
</tbody>
</table>

Table 5.4; Comparison of results using different decarboxylation conditions
(Error bars are standard deviations on triplicate analyses)

The evidence from this experiment is that the 65°C decarboxylation procedure produced slightly higher concentrations of TCAA, which would suggest that abiotic/biotic contributions are accounted for using the normal procedure and that 100°C does not lead to an increased production of CHCl₃. The background CHCl₃ was small for both procedures, but decarboxylation still determined large TCAA concentrations. The larger standard deviation for the 65°C results suggests that the standard procedure is more precise probably because of the shorter preparation time.
The comparison is very time-consuming, but should be repeated on other soils to confirm the findings.

A comparison of results using conditions that do and do not decarbonylate chloral to CHCl₃ must be performed to test whether CHCl₃ thought to originate from TCAA is actually derived from chloral. Köppen et al. (1988) reported that at pH >8 chloral was completely decarbonylated to CHCl₃ within 90 minutes at 60°C. If 1 ml NaOH solution is added to soil samples instead of water then the blank determination during HSGC analysis would include any CHCl₃ from chloral decarbonylation. This would mean that the difference in peak area between blank (60°C) and sample (100°C) vials corresponded to the amount of TCAA in the sample. A comparison between soil TCAA concentrations determined using 1 ml of water and 1 ml NaOH would establish if chloral was present in the sample. This experiment was performed on 4 soils sampled at Dunslair Heights on 14/8/00. Results are shown in Table 5.5.

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>TCAA [ng (g fwt)⁻¹] at neutral pH</th>
<th>TCAA [ng (g fwt)⁻¹] at basic pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO1</td>
<td>7.7 ± 0.2</td>
<td>11.5 ± 1.7</td>
</tr>
<tr>
<td>SO3</td>
<td>6.4 ± 1.0</td>
<td>12.2 ± 2.0</td>
</tr>
<tr>
<td>SO4</td>
<td>197 ± 12</td>
<td>100 ± 24</td>
</tr>
<tr>
<td>SO6</td>
<td>20.0 ± 1.3</td>
<td>29.7 ± 3.0</td>
</tr>
</tbody>
</table>

Table 5.5; Comparison of soil analysis by HSGC at neutral and basic pH

(Error bars are standard deviation on triplicate analyses)

The comparison between results is fairly good and for 3 soils the basic samples produced higher results. However, the largest difference was observed in the soil with the highest TCAA concentration. It is not possible to draw conclusions from such a limited study, but it is probably correct to assume that the concentrations measured in most soil samples are true estimations of the TCAA content. A more detailed comparison by the same approach should be attempted with a variety of soils to determine whether chloral is present in forest soils. Chloral is thought to have an atmospheric source, as an intermediate from the photo-oxidation of 1,1,1-
However, chloral is photolysed rapidly in the atmosphere, with a lifetime of approximately 3 to 4 hours (Rattigan et al. 1993). Therefore it seems very unlikely that large concentrations should be introduced to forest soils via wet deposition. The lifetime of chloral in the soil is unknown, but it is likely that it will be rapidly degraded and is unlikely to be responsible for the concentrations of TCAA measured at Glentress Forest. If the source of chloral was atmospheric then it is questionable whether the peak results should only be detected in soils from tree stands at Dunslair Heights and never in open moorland, forest rides and at Venlaw tree stands during 18 months of sampling.

The suggestion that the soil is best studied using the HSGC technique will undoubtedly cause great debate within the scientific community, but the elucidation of why the HSGC and extraction/derivatisation techniques seem to produce different results is the end goal. The differences found between the two methods offer the opportunity for further detailed research and the chance of co-operation between groups. A comparison of extraction results by base and water extractions, analysed by derivatisation techniques would prove if the same TCAA concentrations could be determined. The large relative differences between samples from different areas of the same site must be investigated to prove that peak concentrations in TCAA exist under tree stands.

**5.5.5 TCAA degradation experiments**

As the soils were analysed within two days of their sampling in this research the possibility that degradation occurs in stored soil over 10 days (Frank et al. 1991) will not alter the results. In practice, more rapid analysis was not possible. Occasionally, what appeared to be rapid TCAA removal in closed vials stored at room temperature has been observed, but with a reported half life of 14 to 90 days (Worthing & Hance 1991) degradation of TCAA is not expected to occur over such a short time. However, the estimates of half-life may depend on the absolute concentrations and may not apply to samples or extracts stored in a glass vial. It is also very unlikely that a single half life for TCAA exists for all soils and soil conditions. The long term storage stability (1 month) of sampled soil and also the degradation of TCAA over
hourly periods for 24 hours at room temperature and 4°C was investigated. The results for the storage of soil over a month is shown in Table 5.6. The bulk soil samples were maintained at 4°C over the storage period, under conditions identical to those used between routine sampling and analysis.

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>Storage time (days)</th>
<th>Concentration at start ng (g dwt)$^{-1}$ TCAA</th>
<th>Concentration at end ng (g dwt)$^{-1}$ TCAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH1 (8/12/99)</td>
<td>7</td>
<td>218 ± 23</td>
<td>211 ± 8</td>
</tr>
<tr>
<td>DH2 (12/1/00)</td>
<td>36</td>
<td>210 ± 19</td>
<td>237 ± 1</td>
</tr>
<tr>
<td>DH5 (14/8/00)</td>
<td>25</td>
<td>584 ± 13</td>
<td>578 ± 11</td>
</tr>
</tbody>
</table>

Table 5.6; Stability of TCAA in soil over a week-month time-scale

(Error ranges are standard deviations on triplicate analyses)

The amount of TCAA present in the soil samples was constant over a period of up to 36 days. This suggests that soils can be stored for analytical purposes, but also that the degradation rate of TCAA in soil is very slow at low temperatures, which has implications for soil as a reservoir and sink for atmospheric TCAA.

A controlled experiment was performed to investigate the degradation of soil TCAA over 24 hours. Occasionally evidence of TCAA degradation was observed in soils stored in a refrigerator over night and this was unsatisfactory for TCAA analysis of soil. Soils (1 g) were weighed into 48 vials and sealed with septa. The vials were split into 2 groups, one of which was maintained at 4°C and the other at room temperature (RT). The group of 24 vials was sub-divided into sets of 4 vials which were exposed to the same conditions. The vials were stored for 0, 1.5, 3, 6, 9 and 21 hours before immediate analysis by HSGC. The temporal trend of soil TCAA concentrations at both temperatures is shown in Figure 5.13.
Figure 5.13; Soil TCAA degradation experiment at room temperature and 4°C

(Error bars are 95% Confidence Intervals of triplicate analyses)

The experiment showed that no significant change in soil TCAA concentrations was detected over a period of 24 hours either after storage at room temperature or in a refrigerator. This finding validates the storage procedure for soil, where analysis is performed within 1-2 days of sampling. The degradation of soil even at room temperature is slow and suggests that TCAA is stable in soil over short periods. The degradation of soils with both high and low TCAA concentrations have been studied and show similar results. The degradation rate of up to 7% per day reported in some soils (Frank et al. 1991) is not representative of the forest soils studied here.

5.5.6 Relationship between trichloroacetic acid and soil properties

The TCAA concentrations of the soil found throughout this study vary greatly, as do the soil properties such as total organic matter (TOM) and water content. By investigating the relationship between TCAA and water content or TOM, evidence of a simple relationship between the soil properties and presence of TCAA might become apparent. Theoretically a relationship between TCAA and TOM may exist if the organic matter is primarily responsible for the natural formation of TCAA. Equally, if the concentration of TCAA in the soil reflects the atmospheric input of
TCAA then with increasing water content increasing TCAA would be expected. This simple approach does not account for the complicated adsorption/binding processes that may occur within the soil nor the rate of water transport through soil relative to water storage capacity. If irreversible binding occurs then the accumulation of TCAA would be reflected by the number of binding sites available and therefore the overall cumulative input rather than the recent input/production. TCAA may be formed by a constituent of TOM such as humic or fulvic acids, which have not been quantified in this study. If leaching of TCAA from the soil occurs then any TCAA from formation processes or atmospheric input is removed quickly and no relationship would be discernible. The routine soil concentrations have been used to produce graphs showing the trend of TCAA with TOM (Figure 5.14) and water content (Figure 5.15). The 10 data points with greater than 80 ng (g fwt)$^{-1}$ TCAA were not included.

The relationships obtained for both graphs are not statistically significant ($R^2 < 0.1$). Figure 5.14 shows that TCAA concentrations are not related to total organic matter, but it is possible TCAA concentration in soil is still be related to some component(s) of organic matter. The current understanding of the formation of TCAA by humic materials in an aquatic environment is outlined by Reckhow et al. (1990). Their conclusion was that aquatic humic acids lead to higher TCAA yield than aquatic fulvic acids. The formation of TCAA instead of CHCl$_3$ is favoured in the presence of an $\alpha$-OH or a conjugated system (Reckhow & Singer 1985) which helps to stabilise the carbonium ion formed upon the oxidative loss of trichloroacetyl. The authors suggest that the degree of conjugation of a fulvic/humic acid can be monitored using the compounds' specific UV absorbance. If a certain component of the organic matter were to be identified as important in soil production of TCAA then this could be routinely isolated and measured for each soil sampled.

Figure 5.15 shows there is also no significant relationship between TCAA and water content of the soil, but that the concentration of TCAA is approximately constant at all water contents of the soil. This would be expected if TCAA were derived solely from natural production from organic matter provided that the organic matter content
Figure 5.14; Relationship between TCAA and total organic content in soil at all sites in Glentress Forest
(Each point represents mean TCAA concentrations in samples analysed in triplicate)

Figure 5.15; Relationship between TCAA and water content in all soils at Glentress Forest
(Each point represents mean TCAA concentrations in samples analysed in triplicate)
of soils was also independent of the water content.

As mentioned in Section 5.5.4.2 the findings of this research suggest that more complicated binding processes may explain the presence of TCAA in soil which therefore complicates any attempt to relate the concentration of TCAA to soil properties. The properties of soils containing high TCAA concentrations may be those that lead to natural TCAA formation processes and/or properties leading to the adsorption of TCAA from soil solution. If the functional groups responsible can be identified then it may be possible to derive a relationship between them and the soil TCAA concentrations.

The soil pH is a further soil property that may be important in the formation of TCAA. It has been reported that the pH of the soil influences the extent of formation of CHCl₃ or TCAA from its intermediates (Hoekstra et al. 1999b). The yield of TCAA is increased at pH <7, whereas more CHCl₃ is produced at pH>8 (Figure 5.1). This simple relationship may be monitored by the measurement of soil pH which could be done routinely without the need for specialist and time-consuming humic acid extractions.

The pH of some soil samples taken at various locations in Glentress Forest and with different TCAA concentrations were measured. The procedure used for pH measurement was that outlined by Dewis & Freitas (1970). The soil samples (2 g fwt) were weighed into screw-cap vials and 5 ml of deionised water added. The vials were shaken vigorously, left to stand overnight and shaken again twice the next day. At 24 hours after the start of the experiment the vials were centrifuged at 4500 rpm for 10 minutes. The supernatant was removed and the pH measured using a pH meter calibrated with buffer solutions. The pH of the soils and the respective TCAA concentrations are shown in Table 5.7.
Table 5.7; pH measurements in various soils from Dunslair Heights

There is no difference in the pH between all the soil samples reported in Table 5.7. This is surprising as some of the samples were taken from beneath tree stands and others were taken from the open moor. Lower pH measurements were expected in soils under coniferous trees, but this has not been observed. Soils with the high and low TCAA concentrations do not have different soil pH and so there is no evidence that high concentrations in these soils occur due to an optimum pH for formation by chloroperoxidase enzymes. The pH range measured in the soils (Table 5.7) is close to the optimum pH of the enzymes (3-5) and are all less than 7, which Hoekstra et al. (1999b) have suggested favours the formation of TCAA. The conclusion from this small study is that pH is not a deciding factor as to whether high TCAA concentrations are present in soil at Dunslair Heights.

5.6 Conclusions

The research on soils described here not only demonstrates large advances in the understanding of TCAA cycling in the soil environment, but also significantly increases the database of TCAA measurements in the literature. The soil compartment has been largely ignored in the past and the measurements which are available have in general been of small sample size and in some cases have been poorly characterised with respect to how the samples have been taken and in what
units the measurements have been quoted (Frank 1988). It was recognised from the start of the project that spatial heterogeneity would make it difficult to obtain reliable quantitative information on TCAA in soil, despite its importance in understanding TCAA concentrations in foliage. For this reason the data set is not as comprehensive as would have been liked. The data do, however, establish a foundation of knowledge for further work and will hopefully stimulate other research interest and debate.

Forest soil has clearly been demonstrated to be a very heterogeneous entity with respect to TCAA concentration. Within tree stands it shows horizontal and vertical variation in TCAA content, which cannot be explained simply. The heterogeneity of the tree stands themselves could be as great a factor as that of the soil underneath it. However, this heterogeneity in TCAA heterogeneity has not been as evident in the open moor soils, which show relatively constant lower concentrations. It is not known whether this stability is due to the lack of natural production or whether the large variation in tree stands is due to huge variability in formation processes over small distances. The differences seen between the types of site monitored is striking. The data set does not establish whether TCAA is being naturally produced or simply accumulated from atmospheric deposition. There is evidence for large TCAA concentrations within tree stands which do not occur at other types of site, but this appears to be related to certain properties present in specific soils within the Dunsclair Heights site, as it is not present at Venlaw which has a different soil type. Simple estimates of the amount of TCAA arising from atmospheric input suggests that another source must be involved. However, this ignores the complicated processes of production and degradation occurring within a forest environment which are not yet fully understood. A simple mass balance for the forest ecosystem, which includes consideration of the findings in this chapter is presented in Chapter 8.

Another important conclusion of this work is that the different analytical methodologies used in the literature may well produce different results for soil TCAA concentrations. The reasons for this inconsistency have been described above, but the different methodologies may be exploited to investigate the soil
processes involving TCAA. If a universal method is adopted for routine analysis then the comparison of results will become easier and more meaningful, permitting greater focus on the processes of binding and production. Previous work (Haiber et al. 1996) suggested that adsorption was suspected to occur, which this work confirms. Identification of the type and nature and the bonding of TCAA to soil is now the goal.

It is still not possible to answer the long standing question as to whether the concentrations present in the needles of trees detected throughout the world is dependent on the soil TCAA levels or more closely related to the atmospheric input. The role of soil has been identified as a major factor in the presence of TCAA in tree needles and its uptake and degradation.

**Summary conclusions**
- TCAA from peaty upland moorland soil is relatively constant at 8 ng (g fwt)$^{-1}$ TCAA.
- TCAA in soil from the lower forested site (Venlaw) has concentrations ranging from 3 to 27 ng TCAA (g fwt)$^{-1}$ with a mean concentration 10 ng (g fwt)$^{-1}$ (n=14), but has no very large values.
- TCAA in soil from the upper forested site has generally larger concentrations with a mean of 75 ng (g fwt)$^{-1}$ [range 6 to 390 ng TCAA (g fwt)$^{-1}$; n=19], but has some very large values in excess of 200 ng TCAA (g fwt)$^{-1}$.
- The very high concentrations of soil TCAA are much greater than calculated due to direct atmospheric input, but were observed in several locations on several dates.
- No relationship was determined between TCAA concentrations in the soil and total organic matter content or the water content.
- Extraction of soil TCAA was very inefficient and is the most likely explanation for the large systematic differences between the TCAA concentrations determined by a direct HSGC analytical method and the extraction/derivatisation techniques.
Chapter 6

Trichloroacetic acid in wet deposition and the gas and particulate phase

6.1 Introduction

It has been assumed that TCAA detected in rainwater stems from photochemical transformation reactions of chlorinated compounds such as 1,1,1-trichloroethane and perchloroethylene (PER) in the atmosphere (Frank et al. 1994; Plümacher & Schröder 1994; Juuti et al. 1993). Hypothetical mechanisms for atmospheric formation processes of TCAA from perchloroethylene have been suggested, which were proposed to proceed through the uptake of trichloroacetaldehyde (chloral) into the aqueous phase (lifetime 20 to 50 days) followed by its oxidation to the final product (Gay et al. 1976). The aqueous oxidation process is in competition with the rapid photolysis of chloral in the atmosphere ($\tau = 3-4$ hours) and so is only a minor product of the reaction (Franklin 1995). Despite these hypotheses, no relationship between chlorinated solvent and TCAA concentrations in rainwater has been proved. Nevertheless TCAA is expected to exist in the atmosphere almost exclusively in the aqueous phase, due to its high hydrophilicity and its low vapour pressure (120 Pa at 50°C) (Daubert & Danner 1989-1991). The ease of sampling and analysis of wet deposition means that the study of TCAA in this matrix is widespread in the literature (see references in Table 6.1). The measurement of gas or particulate phase TCAA has been less well studied partly because low concentrations are expected and partly because of the lack of an established analytical methodology.

The concentration of TCAA in wet deposition is also low, generally in the sub-part per billion level. This means a large volume of sample is required and possibly a pre-concentration step to exceed analytical detection limits. Two approaches to sample pre-treatment were described by Reimann et al. (1996). An evaporation method took a sample to dryness before derivatisation with propanol/ sulphuric acid and analysis by GC-ECD. The second method used a strong anion exchange resin to concentrate TCAA, with subsequent elution and esterification with the same reagent.
It has been noted that when a strong anion-exchanger is used the recovery of TCAA can be difficult (Grimvall *Pers. comm.*). The other common method was described by Frank *et al.* (1990), which involved the extraction of TCAA into solvent, followed by derivatisation with diazomethane. These three methods have in common an extensive pre-treatment followed by derivatisation and analysis, which at such trace concentrations may lead to possible contamination and losses unless extreme care is taken. The other more direct method used by Schleyer *et al.* (1996) was HSGC. This protocol seems to be custom-made for precipitation analysis as the only handling required is to pipette an accurate volume into a vial, previously heated and cooled to drive off background CHCl₃. The question mark with this methodology is its sensitivity. All of the methods are more straightforward with a simple matrix such as precipitation than with soil or needles.

The concentration of TCAA in rain has been extensively measured throughout Central Europe in the 1990s. The literature values are summarised in Table 6.1. For more detailed information see the associated references.

The work by Reimann *et al.* (1996) was carried out at an urban site in Zurich and two sites in the surrounding rural area. The temporal pattern of concentrations shows that maxima were observed in both August 1994 and June 1995, suggesting an increased atmospheric formation process in the summer months. No correlation was found between measured concentrations and rain volume, the duration of the preceding dry period or the volume of the most recent rain event. However, a correlation was found between the TCAA concentration and the solar radiation experienced over the preceding days, which led to the suggestion that chloroacetic acids were formed in the atmosphere by direct or indirect photochemical reaction as the rate limiting step. This suggestion was also thought to explain the different concentrations measured in the summer and winter months. A comparison of the concentrations from the rural and urban sites showed no significant difference between the sites. At the rural site a comparison of concentrations in rain collected from either open fields or as throughfall showed higher concentrations in the latter. It was suggested that this was
due to TCAA present on the needle surfaces from dry deposition, which was rinsing off in rain events.

<table>
<thead>
<tr>
<th>Sampling site/ date</th>
<th>Conc. Range [ppb]</th>
<th>Mean conc. [ppb]</th>
<th>n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hessen, Germany 1988-89</td>
<td>0.2 - 6.5</td>
<td>1.7</td>
<td>94</td>
<td>Schleyer et al. (1991)</td>
</tr>
<tr>
<td>Berlin, Germany 1990-91</td>
<td>0.1 - 20.0</td>
<td>2.1</td>
<td></td>
<td>Plümacher (1995)</td>
</tr>
<tr>
<td>Bonn, Germany 1990-91</td>
<td>(max. 7.5)</td>
<td>0.8</td>
<td>(0.25)</td>
<td>Schöler et al. (1991)</td>
</tr>
<tr>
<td>Siegen, Germany 1991-92</td>
<td>0.1 - 2.0</td>
<td>0.57</td>
<td></td>
<td>Haiber et al. (1996)</td>
</tr>
<tr>
<td>Hau, Bonn, Germany Jan-Nov 1992</td>
<td>0.08 - 0.3</td>
<td>0.15</td>
<td>(0.12)</td>
<td>Haiber et al. (1996)</td>
</tr>
<tr>
<td>Bleche, Germany 1991-93</td>
<td>0.05 - 9.7</td>
<td>(0.16)</td>
<td></td>
<td>Clemens (1993)</td>
</tr>
<tr>
<td>Austria 1991-93</td>
<td>0.01 - 0.3</td>
<td></td>
<td>85</td>
<td>Lorbeer et al. (1994)</td>
</tr>
<tr>
<td>Switzerland 1993</td>
<td>0.03 - 0.9</td>
<td>0.3</td>
<td>44</td>
<td>Müller et al. (1996)</td>
</tr>
<tr>
<td>Switzerland 1993</td>
<td>0.044 - 0.71</td>
<td>0.3</td>
<td></td>
<td>Reimann et al. (1996)</td>
</tr>
<tr>
<td>Switzerland 1993-95</td>
<td>0.13</td>
<td></td>
<td></td>
<td>Reimann et al. (1996)</td>
</tr>
<tr>
<td>Germany (open field) 1989-91</td>
<td>0.02 - 3.27</td>
<td>(0.44)</td>
<td>34</td>
<td>Schleyer et al. (1996)</td>
</tr>
<tr>
<td>Germany (open field) 1993-94</td>
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<td>29</td>
<td>Schleyer et al. (1996)</td>
</tr>
<tr>
<td>Germany (throughfall) 1989-91</td>
<td>0.69 - 6.85</td>
<td>(2.68)</td>
<td>35</td>
<td>Schleyer et al. 1996</td>
</tr>
<tr>
<td>Germany (throughfall) 1993-94</td>
<td>0.55 - 2.33</td>
<td>(0.63)</td>
<td>30</td>
<td>Schleyer et al. 1996</td>
</tr>
<tr>
<td>Kuopio, Finland Feb 94-Nov 95</td>
<td>0.02 - 210</td>
<td>0.12</td>
<td></td>
<td>Juuti et al. 1997</td>
</tr>
</tbody>
</table>

Table 6.1: Literature TCAA rain concentrations at European sites

|median values in parentheses|

Müller et al. (1996) also studied rain TCAA concentrations in Switzerland between January and December 1993 and from March to August 1993 at sites in Dübendorf and Zurich. The mean and the range of the concentrations were very similar at the two sites despite both sites rarely experiencing the same rain event. It was suggested that the concentration determined was not correlated with rain intensity, and that there was a seasonal variation of TCAA with higher concentrations generally found
during the summer months. Five possible explanations were put forward; (1) smaller direct input of TCAA into atmosphere in the winter, (2) lower atmospheric concentrations of chlorinated precursors in winter, (3) reduced photolysis in winter, (4) greater precipitation in winter, (5) different phase-exchange capabilities during washout. It seemed that there was unlikely to be a direct input of TCAA into the atmosphere due to its low volatility and high Henry's Law constant ($K'_H = 7.4 \times 10^4$ mol kg$^{-1}$ atm$^{-1}$ at 298 K). The lower temperature during winter months could lead to significant changes in the atmospheric chlorinated solvent concentrations due to lower fugitive emissions, changes in vapour pressure or even by dilution. The reduced photolysis is important as is the greater amount of precipitation, but the latter may be corrected for by using a rainfall weighted mean concentration. The exact mechanism for formation of TCAA in the atmosphere is uncertain, with the aqueous oxidation of perchloroethylene and methyl chloroform not widely accepted, so it is hard to be precise as to what causes the seasonal variations that are found at certain locations.

The pattern of seasonal variation with maxima in the summer has been reported by several authors, but is not seen in all studies. Haiber et al. (1996) reported a summer maximum at Siegen, Germany where the rain TCAA concentration varied from 0.1 ppb during November and December to 2.0 ppb in June 1992. This seasonal pattern was also reported by Fillibeck et al. (1995). During the same research of Haiber et al. (1996) at Hau, not far from the site at Siegen, no seasonal variation was observed. This was explained by the contribution of other unspecified TCAA formation processes, which did not depend on photochemical or biochemical activity. The variation of the seasonal average concentration between the two sites was large with 570 ppt at Siegen and only 140 ppt at Hau. This difference was suggested to be due to the transport of pollutants from the industrial city of Siegen to the sampling site, a source which was absent at Hau. In another study Clemens (1993) found that at Bleche, Germany there was a uniform input of TCAA from April 1991 to February 1993 with the exception of a large event in November 1991. The absence of a seasonal maximum was attributed to the rural nature of the site. The logical consequence of this hypothesis is that the formation of TCAA is enhanced in the
summer months in urban areas. However, Reimann et al. (1996) reported that urban and rural measurements were not significantly different within and outside Zurich. It is possible that the urban areas could act as source of chlorinated solvent emissions especially from dry cleaning sources, but it is not clear how this would affect the TCAA concentrations in rain. It is still not known how quickly TCAA might be formed from its possible precursor compounds and it is likely that these precursors might be dispersed and the concentrations averaged in the atmosphere by dilution leading to few such peak concentrations. Seasonal variations in rain TCAA concentrations might also be significantly affected by the differences in precipitation volume and frequency specific to each site, especially if they were geographically discrete from each other.

It has been suggested that the first volumes of rain from an air mass contain the highest concentrations of haloacetic acids, with subsequent washout and decreased concentrations with time (Scott et al. 1999; Berg et al. 2000). This would suggest that the measurements of TCAA concentrations in the same air mass over time would highlight the behaviour and formation of TCAA and how it is rained out. Sampling of discrete temporal samples during a rain event would test this hypothesis of decreasing concentrations. Geographical differences might also influence TCAA concentrations. For example, precipitation sampled in Great Britain from westerly trajectories might indicate TCAA being rained out as the air mass progresses eastwards giving lower concentrations in Central Europe. A more sophisticated monitoring programme would distinguish different rain events and relate them to their trajectory. As most measurements in the literature have been made on pooled weekly samples this is not possible. The results from Continental Europe suggest comparable concentrations throughout, though the areas of study tend to be concentrated in Germany, Austria and Switzerland.

Few measurements of TCAA in wet deposition have been carried out on long time scales. This type of study is of particular interest as the comparison between the trend in TCAA concentrations and the decreasing chlorinated solvent emissions might demonstrate a causal link between source and pollutant. The only study to
investigate this was by Schleyer et al. (1996) carried out in 1989/91 and 1993/94. The results (Table 6.1) suggest that the concentration of TCAA in wet deposition collected in both the open field and as throughfall has decreased between the two periods. However, this was not related to the concentrations of atmospheric chlorinated solvents such as 1,1,1-trichloroethane or PER, and so no relationship between precursor and TCAA was offered.

The wet deposition measurements in the literature have almost exclusively been limited to rain sampling. This is the easiest medium to sample, but ignores the contribution and importance of wet input from cloud. It is known that cloud can scavenge pollutants from the atmosphere and it has been reported that many ionic species, including the major pollutants, are more concentrated in cloudwater than rain (Gervat et al. 1985; Fowler et al. 1988; Schmitt 1988; Prinz & Krause 1989). Crossley et al. (1992) reported that, although the cloudwater deposition represents only 25% of the hydrological input at Glentress Forest, the annual input of pollutants by cloud is between 2 and 4 times greater than by rainfall. The significance of this is greater where surface vegetation is efficient at capturing cloudwater, such as in forests. The importance of cloud to a forest ecosystem will not only be in the overall magnitude of the inputs, but also the frequency and duration of the exposure of the trees to elevated concentrations. Although the overall input may not be harmful to the trees, the high concentrations in cloud events may cause physiological damage on exposure. With the known phytotoxic properties of TCAA it is surprising that cloudwater has not been more thoroughly studied.

There have been few gas-phase measurements of TCAA. It is assumed that because of the high solubility and hydrophilicity of TCAA it exists almost exclusively in the aqueous phase. Due to the uncertainty about its formation process it is not known whether TCAA is formed either in the gas phase with subsequent dissolution or directly in the aqueous phase. Studies of the temporal variation of gas-phase TCAA might help to resolve these questions.
Only two published measurement studies of TCAA in air exist, both by the same author. Among the sampling methodologies considered were cryotrap and adsorption cartridges, but Frank et al. (1995) employed denuders which had low flow resistance and an ability to sample both gas-phase and particulate-bound TCAA. The preparation of the denuders for sampling was slow and involved the internal coating of six straight glass tubes (500 x 8 mm) with a saturated solution of Na$_2$CO$_3$ in glycerol and leaving them to dry overnight. For sampling these tubes were connected in series with ground-glass joints to a diaphragm membrane pump at a rate of 3 l min$^{-1}$. After a total volume of 10 m$^3$ was sampled the coating of the denuders was eluted with water and the TCAA determined using a derivatisation method. A blank denuder tube was kept sealed concurrently during sampling. Breakthrough experiments demonstrated that the denuders trapped 80% of a spiked sample of haloacettes within the first 50 cm tubing and 95% within 1 metre.

This methodology clearly works well, but the time taken to sample the required air volume (10 m$^3$) is 3 days. It was noted by Frank et al. (1995) that not much was known about the TCAA concentrations in air due to insufficient sensitivity of the methodology, and stated that the concentrations were expected to be in the ppt to ppb range. Sampling of such a low concentration required the analytical method to have a very low detection limit and exceptionally low blank levels. The expected minimum concentrations corresponded to about 6 ng m$^{-3}$ (1 ppt), although calibration was performed between 0.1 and 100 ng sample$^{-1}$ (10 m$^3$ air). The sampling took place at Tübingen University during December 1993 and April and June 1994. The reported results for TCAA were all quoted as < 300 pg m$^{-3}$ and were significantly lower than other haloacetic acid concentrations in air and rain at that site. The second sampling programme was performed at the southern edge of Schönbuch Forest near Tübingen between March and August 1992 (Frank et al. 1994). The range of TCAA concentrations found there were 327 to 2614 pg m$^{-3}$. This seemed to indicate that TCAA concentrations in air was higher in forested areas than in urban areas, but the validity of these large differences should be questioned since no other measurements have been made. It is very hard to draw conclusions from such limited data, but clearly the concentrations of TCAA in air are very small.
6.2 Sampling programme

The sampling of wet deposition has been carried out since 1987 at Glentress Forest as part of the measurements of acid deposition carried out by The Centre for Ecology and Hydrology (CEH). Samples of cloud and rain were collected at Dunsclair Heights and rain only at Venlaw, which were subsequently analysed for major ions. Stored reference samples were available for TCAA analysis, having been maintained at <4°C to stop biological activity. For a full description of the sampling programme see Crossley et al. (1992 & 1998).

Two sites were chosen for the sampling and monitoring of TCAA in air, one was at Dunsclair Heights in Glentress Forest to obtain measurements at a forested site and the other at the Department of Chemistry, University of Edinburgh to obtain complementary urban data. The air sampling programme was carried out from March 1999 to April 2000.

The aims of the wet deposition and air sampling programme were:
- To obtain complementary wet deposition and air TCAA concentration data for the Glentress Forest site concurrent with other monitoring programmes (Chapters 4 & 5).
- To investigate seasonal trends of TCAA in wet deposition and air data for comparison with literature values for European sites.
- To compare the TCAA concentrations of cloud and rain at Dunsclair Heights.
- To compare and contrast wet deposition inputs and concentrations at both sites within Glentress Forest for use in mass balance calculations (Chapter 8).
- To compare air TCAA concentrations at an urban and rural site.

6.3 Experimental

6.3.1 Sampling protocol

6.3.1.1 Rainwater sampling

A 200 mm diameter Pyrex glass funnel, set 1.5 m above ground level and draining into a polypropylene bottle, was used for weekly collection of rain. The volume of rain was determined by weighing.
6.3.1.2 Cloudwater sampling

A conical passive 'Harp-wire' device was used, developed from that described by Dollard *et al.* (1983) based on an original concept by May (1961). This comprised a polypropylene disc 12 mm thick, machined to provide teeth (3 mm apart) around its circumference, set on a stainless steel frame (coated with low density polypropylene) so the base of the frame formed an opening to an inverted cone (figure 6.1). Initially, the collector was strung with a PTFE (Tefzel, Du Pont) (0.55 mm diameter) coated copper wire, but this was later replaced by polypropylene monofilament (0.6 mm diameter) running as a continuous strand between successive teeth and the apex ring.

To protect the cloud collector from rainfall, a 1.2 m diameter polypropylene faced lid was mounted over the collector on a framework that also housed the funnel and bottle (Unsworth & Crossley 1987). This lid excluded raindrops larger than 0.5 mm in diameter when the windspeed was less than 5 m s⁻¹. Cloud droplet collection relied on the ambient airflow impaction on the filaments, with a component of the windforce accelerating the droplets down the strings into the collecting funnel/bottle. The volume of cloudwater was determined by weighing. The calibration of the cloud collector is outlined by Crossley *et al.* (1992), who determined the collection efficiency of the collector \( (C_d) \) to be 0.29. To determine the input of TCAA to a forest by cloud the deposition recorded by the cloud detector was multiplied by the capture efficiency of the forest (0.06), as determined by Beswick *et al.* (1991), and divided by the capture efficiency of the gauge (0.29, calibrated).

6.3.1.3 Air sampling

The procedure used in this work for air sampling of TCAA is a novel technique using the affinity of the acidic TCAA for Na₂CO₃ solution. Air was sampled through Na₂CO₃ impregnated filters held in an open-faced filter holder. The denuder method (Frank *et al.* 1995) was time-consuming and not suited to use in the field.

Glass microfibre filters (Type A/C; Pall Gelman) were used, as these were found to contain the lowest background CHCl₃ levels. This was a vital parameter for air sampling as background CHCl₃ could potentially obscure the TCAA sampled from
the air. The filters were soaked in 0.1 M Na$_2$CO$_3$ solution before drying in air. The preparation time was 5 minutes for impregnation, plus drying time which varied, but was generally in the time-scale of a few hours. The filters were prepared in a batch mode with 6 filters prepared each week (4 for sampling and 2 blanks). After preparation the blank filters were immediately transferred to a sealed pre-heated headspace vial and stored in a refrigerator. Using tweezers the sample filters were mounted in open-faced filter holders (polycarbonate or aluminium) according to their sampling site.

At Dunslair Heights two aluminium filter holders were mounted on a stainless steel plate. The plate could be screwed onto the sampling shroud quickly and simply, and by using two plates and 4 holders it allowed on-line filter change each week. The filter holders were capped before and after use and during transport to stop contamination.

The sampling apparatus used at Dunslair Heights was a modification of an active cloud sampler used by CEH and comprised a rectangular, perspex box with a ground-facing inlet at one end and an in-built fan mechanism at the other. It was mounted on a tripod 1.5 m above the ground (see Figure 6.2). The box contained a set of polypropylene strings to remove any cloud moisture from the air flow which was maintained as laminar flow using a honeycomb grid at the inlet. For TCAA air sampling the fan mechanism was removed and an aluminium filter plate was attached using nuts, which ensured intake of air was only possible from the front inlet. A mechanical pump (sampling rate 20-21 l min$^{-1}$) housed in a waterproof box was connected in series to the filter plate and a mass flow gas meter. The tubing from the pump to the plate was split using a ‘T connector’, allowing parallel sampling through each filter. Each section of tubing from the ‘T’ to the filter holder was of the same internal diameter and length to give equal flow through each filter. It was assumed that the total volume of air was drawn equally through each filter.

At the urban site in Edinburgh polycarbonate filter holders were used. Two filters were arranged in parallel, connected in series to a pump (sampling rate 13-14 l min$^{-1}$)
Figure 6.1; Passive cloud sampler at Dunslair Heights

Figure 6.2; Air sampling apparatus at Dunslair Heights
and a gas meter, as described above. The filter holders were positioned in a window of the chemistry department to allow sampling of external air without direct impact of rain or moisture. An added feature of this setup was the ability to use a PTFE filter (1μm pore size) in front of and spatially separated from, the back Na₂CO₃ filter, which meant that a distinction between particulate bound and gas-phase TCAA could be made.

At both sites side-by-side sampling was performed enabling duplicate weekly measurements. The sampling rate achieved using this method was high (15 to 20 l min⁻¹) without any breakthrough of sample (Section 6.3.3.1), which gave the advantage of being able to sample large volumes of air in one week periods (up to 200 m³). The limiting factor on the sampling rate was the pump capacity. Frank et al. (1995) demonstrated that the expected air concentration of TCAA was in the 0 to 300 pg m⁻³ range and so the ability to quickly and reproducibly sample large volumes of air was necessary if the detection limit of the method was to be reached.

6.3.2 HSGC analysis

6.3.2.1 Rain and cloud water
Precipitation was analysed by automatic Headspace Gas Chromatography as outlined in Chapter 3. The analysis of aqueous samples is far simpler than for solid matrices e.g. soil, due to the limited sample pre-treatment required. Matrix matching of the samples and standards enabled calibration using aqueous TCAA standards without the need for estimating partition ratios.

Initial experiments used a sample volume of 1 ml of rain or cloud, but this showed insufficient sensitivity and was only subsequently used if the volume of available sample was low.

From the filtered precipitation sample a sub-sample was transferred into a glass headspace vial (25 ml) previously heated and cooled to drive off any CHCl₃. This sample was degassed with oxygen-free nitrogen (OFN) for 1 hour to remove any background CHCl₃. Using auto-pipettes 5.00 g (± 0.01 g) aliquots of the sample
were transferred to pre-heated glass headspace vials and were crimped with PTFE coated rubber septa. Vials were prepared in quadruplicate to allow 3 TCAA sample and 1 blank CHCl₃ determinations. Unless the samples were to be analysed immediately the sample vials were kept in a freezer to stop any possible breakdown of the analyte.

When TCAA was to be determined the vials were put through the procedure of decarboxylation and HSGC analysis using the conditions shown in Table 3.4. The blank vials were heated to 60°C only in order to determine background CHCl₃ in the samples. The results were calculated as outlined in Section 3.3 using a partition ratio for 5 g of precipitation of 1.00 (i.e. no partitioning).

6.3.2.2 Air samples

6.3.2.2.1 Manual method

The analysis of the majority of air samples has been performed using a manual HSGC method as outlined in Section 3.4.1. Although very time consuming this method was the most difficult to automate due to the inability to perform a blank CHCl₃ correction using a replicate sample. Whereas for other matrices an identical sample vial could be prepared, which was analysed for residual CHCl₃, the sampled air filters were unique, and different from the initial blank filters. For this reason blank CHCl₃ had to be determined on the actual sample filters before the analysis for TCAA. This required the equilibration of vials at 60°C and injection of an aliquot of headspace before decarboxylation at 100°C. After decarboxylation the vials were re-equilibrated at 60°C and the headspace re-analysed. The crucial requirement was that the septum could be pierced, and subsequently heated to 100°C without the loss of any analyte. This was not possible with the automatic method as the holes produced were not self-sealing. However, the use of self-sealing septa and syringes with fine needles meant that the manual method was suitable.

The manual HSGC parameters (Table 6.2) were a combination of those determined to be optimum in Section 3.4.1. A decarboxylation time of 90 minutes was used to achieve uniformity between the automatic and manual analysis in all matrices.
The air filters were equilibrated at 60°C for 1 hour before injection of headspace sample into the GC. The blank CHCl₃ areas for all the vials were determined before decarboxylation for 90 minutes and re-equilibration. The final CHCl₃ peak areas were assumed to be a combination of background CHCl₃ in the sample and CHCl₃ from decarboxylated TCAA. By subtraction of the blank area from the sample area the concentration of TCAA in the air filter was calculated. It was also necessary to correct for any TCAA present in the blank impregnated filters, perhaps from the Na₂CO₃ solution. The calculation is shown in Equation 6.1. Calibration was performed used external TCAA standards in water. Each standard vial contained approximately 5 ng TCAA in 200 µl volume, and was analysed every 6 injections.

C_N = (Area_{sample} - Area_{blank}) \cdot f_w^{-1} \cdot v_{air}^{-1} \quad \text{Equation 6.1}

where:

- \( C_N \) = concentration of TCAA in air (pg m⁻³)
- \( Area_{sample} \) = peak area of TCAA in sample air filter
- \( Area_{blank} \) = peak area of TCAA in blank air filter
- \( f_w \) = actual response factor of TCAA in water
- \( v_{air} \) = volume of air sampled

### 6.3.2.2.2 Automatic method

The automation of the method described in Section 6.3.2.2.1 was essential as the manual method became very time consuming and involved periodically changing the setup of the instrument. The problem existed in the determination of the blank
CHCl₃ areas of the samples, which was not possible automatically. The solution was found by heating the air filters in open vials prior to crimping. By heating at 60°C for 45 minutes any background CHCl₃ was desorbed from the filters without any decarboxylation of TCAA. This was performed immediately after air sampling ceased, so enabling the filters to be stored until analysis.

The automated HSGC analysis of the samples used the same parameters as outlined in Table 3.4 except that the split flow was 10 ml min⁻¹. Calibration was performed using external TCAA standards containing 5 ng TCAA in a 200 µl volume, which were analysed every 6 injections. The concentrations of TCAA in the sampled air was calculated using Equation 6.1.

6.3.3 Initial development results

6.3.3.1 Breakthrough experiments

The development of a method of air sampling traditionally requires the determination of a breakthrough volume. This is the maximum volume of air that may be sampled before the method stops efficiently trapping the analyte of interest. It is essential that the method efficiently captures the analyte of interest in order to accurately determine the air concentrations. A standard method of determining breakthrough is to sample a known volume of air with a known concentration of analyte, often produced by a standard gas generator. This is good for a method using a sample probe or tube which draws sample into the trapping matrix, but is not feasible for an open-faced sampler.

To test breakthrough with the air sampling method developed here it was possible to use two impregnated filters in series, one behind the other. If the first filter was efficiently capturing TCAA from the air the second filter would show no TCAA concentration. It was found that if the filters were in physical contact with each other it was apparently possible to transfer some TCAA from the front filter. It was for this reason that the filters were spatially separated by a 1 cm gap within the polycarbonate open-faced filter holders to test whether gaseous TCAA could penetrate the front filter. The results are shown in Table 6.3 with IF and IB.
referring to the front and back filters of holder #1 etc. Filters 1 and 2 are collected from parallel sampling.

<table>
<thead>
<tr>
<th>Sampling date (vol. of air)</th>
<th>Trichloroacetic acid in air (pg m(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/6/99 (135)</td>
<td>1 F: nd, 1B: nd, 2F: 2.3, 2B: 0.6</td>
</tr>
<tr>
<td>9/6/99 (82)</td>
<td></td>
</tr>
<tr>
<td>30/6/99 (72)</td>
<td></td>
</tr>
<tr>
<td>4/7/99 (95)</td>
<td></td>
</tr>
<tr>
<td>9/7/99 (115)</td>
<td></td>
</tr>
<tr>
<td>15/7/99 (122)</td>
<td></td>
</tr>
<tr>
<td>21/7/99 (137)</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.3; TCAA in air results from breakthrough experiments 
\(n.d = \text{not detected}\)

The results fit into two phases of development of the method. The initial results from 2/6/99 to 30/6/99 consistently determined TCAA on both the back and front filters suggesting breakthrough to the back filters. It was discovered that more care was needed in the attachment of the front filters as small gaps were allowing air to bypass the front filter. The subsequent results show ideal results with no TCAA detected and hence no significant breakthrough to the back filters of seven consecutive samples. The trapping efficiency shows no difference whether the sample volumes were low (95 m\(^3\)) or high (137 m\(^3\)), which means that at least 1 week sampling periods were possible. It should be noted that these volumes approximately 10 to 15 times greater than those sampled by Frank et al. (1995), by using a faster sampling rate. This sampling method is expected to give an accurate determination of TCAA in the air. This experiment also indicates that TCAA present on the filter or adsorbed on particulate cannot be released from the front filter to the back one. It has been suggested (Barrie, L; Pers. Comm.) that TCAA on particulate matter may be volatilised and will pass onto the back filter and be determined as gas-phase TCAA, but this does not appear to be the case for this method (Section 6.3.3.2).
6.3.3.2 Contribution of particulate-bound and gas phase TCAA

Development work was performed to determine whether TCAA found in air was predominantly associated with particulate matter or whether it was present in the gas phase. This was determined using a similar method to the breakthrough experiment with the difference being that a 1.0 µm PTFE filter was used as the front filter. This filter trapped all but the finest particulate matter allowing gas phase material to pass through to the impregnated filter behind. It should be noted that although the efficiency of the PTFE filter to capture particulate is not being determined, and is unlikely to be 100%, an assessment of the contribution of each phase to the total TCAA in air can be attempted. Results are shown in Table 6.4.

<table>
<thead>
<tr>
<th>Sampling date (vol. of air)</th>
<th>Trichloroacetic acid in air (pg m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1F</td>
</tr>
<tr>
<td>26/5/99 (141)</td>
<td>23</td>
</tr>
<tr>
<td>13/6/99 (188)</td>
<td>8</td>
</tr>
<tr>
<td>23/6/99 (143)</td>
<td>nd</td>
</tr>
<tr>
<td>28/7/99 (143)</td>
<td>147</td>
</tr>
</tbody>
</table>

Table 6.4; Gas phase and particulate-bound TCAA in air

\[n.d = \text{not detected}\]

The results (Table 6.4) suggest that the contribution of the particulate-bound material to the total TCAA burden in air is small. The first three results show that negligible particulate-bound TCAA is present, but the final sample shows both high particulate and gas-phase concentrations, which also illustrates the temporal variability in atmospheric TCAA concentrations. The fact that both particulate and gas phase concentrations are high suggests that a dirty air mass or large pollution event was experienced. If high particulate TCAA occurred when gas phase concentrations were low, a combined total TCAA value would lead to anomalous, high results. It was decided that urban site in Edinburgh would continue to sample both gas phase and particulate-bound TCAA separately. Another useful measurement would have been to use pre-weighed PTFE filters to determine the total mass of particulate
sampled, leading to a mass of TCAA as a proportion of total particulate. However, the equipment to do this was not available and so only a rough assessment of TCAA in particulate was possible.

### 6.3.3.3 Variations in sampling rate

The use of two filters in the open faced filter holders is bound to change the characteristics of the sampling, so consideration was given to whether the use of two filters with the front one gradually building up with particulate would affect the sampling rate through the filters. Periodic measurement of the flow meter readings during sampling gave an assessment of the variation in flow rate with time. The results shown in Table 6.5 are for sampling commenced on 9/6/99. At the end of the sampling period 82 m³ of air had been sampled and the front filter was described as 'black'. It appears that the flow rate was very stable over the sampling period despite the build up of particulate on the front filter, which means there are no implications of using this protocol for this sampling procedure.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate</td>
<td>13.6</td>
<td>13.6</td>
<td>13.8</td>
<td>13.6</td>
<td>13.6</td>
</tr>
</tbody>
</table>

**Table 6.5; Variation in flow rate with sampling time**

### 6.3.3.4 Detection and quantitation limits

It had been assumed from the start of this work that interferences in the method would be primarily from background CHCl₃ present in the filter samples. This contribution proved to be consistent and though significant, still quite small. A more important interference comes from background TCAA present in an impregnated filter. The impregnation of the glass micro-fibre filters with a solution of 0.1 M Na₂CO₃ solution potentially introduces TCAA to the filters, but as the solution is made up with HPLC-grade water there is no way to minimise it further. For this reason two blank filters are prepared with each set of sample filters and used to determine the average blank TCAA concentration. It is this contribution that
varies with each filter preparation, which determines the detection limit of the method. For this reason only a generalised discussion can be made here. The best and worse cases of blank determinations are shown in Table 6.6.

<table>
<thead>
<tr>
<th>Blank filter date</th>
<th>15/8/99</th>
<th>29/9/99</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCAA (ng)</td>
<td>0.75, 0.95</td>
<td>1.92, 3.76</td>
</tr>
<tr>
<td>Mean ng TCAA (μ)</td>
<td>0.85</td>
<td>2.84</td>
</tr>
<tr>
<td>Blank std. dev (σ)</td>
<td>0.14</td>
<td>1.30</td>
</tr>
<tr>
<td>LOD (ng) = μ + 3σ</td>
<td>1.27</td>
<td>6.74</td>
</tr>
<tr>
<td>LOQ (ng) = μ + 6σ</td>
<td>1.69</td>
<td>10.64</td>
</tr>
</tbody>
</table>

Table 6.6; Limits of detection and quantitation for sampling TCAA in air

The best case scenario shows both low TCAA concentrations and small standard deviation. This enables the accurate estimation of the contribution of the blank and hence a low LOD and LOQ. The worst case scenario is the reverse case with a high standard deviation as the problem. The absolute detection limit is quoted in nanograms of TCAA so the volume of sample must be considered. At Dunsahir Heights a weekly sample was normally 220 m$^3$ whereas in Edinburgh it was lower, at about 140 m$^3$. The best and worst case LOD/ LOQs calculated as a volume concentration are shown in Table 6.7.

<table>
<thead>
<tr>
<th></th>
<th>Dunsahir Heights</th>
<th>Edinburgh</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (pg m$^{-3}$)</td>
<td>12 - 61</td>
<td>18 - 96</td>
</tr>
<tr>
<td>LOQ (pg m$^{-3}$)</td>
<td>15 - 97</td>
<td>24 - 152</td>
</tr>
</tbody>
</table>

Table 6.7; Ranges of LOD and LOQ for air sampling

It is very important to prepare low and reproducible blanks for the accurate determination of TCAA in air. Generally the blanks were reproducible and in the range of 1 to 2.5 ng per filter. Table 6.8 shows the results from an experiment in which a set of 4 filter blanks were prepared and analysed, enabling reproducibility to be estimated for blank preparation. The 6% RSD obtained is excellent at such low concentrations and validates the method of preparation of the filters.
### Table 6.8: Blank filter reproducibility

<table>
<thead>
<tr>
<th>Blank filter date</th>
<th>14/8/00</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCAA (ng)</td>
<td>1.63, 1.56, 1.45, 1.66</td>
</tr>
<tr>
<td>Mean TCAA (ng)</td>
<td>1.58</td>
</tr>
<tr>
<td>Blank std. dev (σ)</td>
<td>0.09</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>5.9</td>
</tr>
</tbody>
</table>

#### 6.3.3.5 Validation of automatic HSGC method

As outlined in Section 6.3.2.2.2 the automation of the air sampling method was difficult because of the need to determine both the CHCl₃ blank concentration and the TCAA concentration on the same sample. To avoid this open vials containing the air filters were heated for 45 minutes at 60°C to remove any CHCl₃ before sealing the vial and subsequently analysing it for TCAA. To validate this procedure 8 blank filters were impregnated with Na₂CO₃ solution, of which half were heated in a fan oven and half in a box oven. The vials were then sealed and analysed for CHCl₃. After analysis the vials were sealed with a new septum, decarboxylated and analysed for TCAA. The results are shown in Table 6.9.

<table>
<thead>
<tr>
<th></th>
<th>Hot box oven</th>
<th>Fan oven</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blank area</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Mean TCAA area</td>
<td>110981</td>
<td>115305</td>
</tr>
<tr>
<td>Std deviation</td>
<td>15949</td>
<td>19568</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>TCAA (ng per filter)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 6.9: Results from filter heating tests

The most important result from the experiment is that CHCl₃ can be removed from the filters to an undetectable level in 45 minutes. This suggests that the same should be possible for real air sample filters. The absolute amount of TCAA on the filters appears to be the same by both methods, at about 1 ng per filter. This is a very low level and is adequate for air sampling. In practice the air filters were heated in a fan
oven as being the most controllable method, with a good circulation of air to remove CHCl₃.

6.4 Results

6.4.1 Rain and cloud

6.4.1.1 TCAA concentration

The concentrations for Dunslair Heights rain (DHR), Dunslair Heights cloud (DHC) and Venlaw rain (VR) over the period June 1998 to May 2000 are shown in Figure 6.3. This data shows the trends in TCAA concentrations including any gaps where no sample was available due to insufficient volume or where no sample was collected.

The main feature of the three data sets is the huge peak in cloud TCAA concentration in May 1999. This is the maximum concentration determined over the 2 year period in all three sample types. This peak in cloud concentration is not accompanied by maxima in rain concentrations in DHR and VR. This enhancement in cloud TCAA concentrations can be detected at various times throughout the sampling period notably in June 98, March and April 99, June 99, August 99 and March 00. The concentration factor between cloud and rain is generally in the order of 2:1, but occasionally is as much as 7:1 (May 99 and June 98). It has been reported that cloud concentrates anions (Crossley et al. 1992 & 1998), so this finding is of no surprise and may prove to be important for tree health as cloud events of high concentration may be more damaging than chronic exposure to lower concentrations of TCAA. The peak concentrations tend to occur in the spring and summer months, so it is possible that they are due to increased TCAA production from photolysis, but it may also be due to volume effects with more concentrated, low volume events in the drier months. There are also problems associated with sampling at a fixed sampling point in relation to the cloud base. Close to the cloud base the concentrations are very high, but these are diluted with height within the cloud as water droplets grow in size. A fixed sampling point collects cloudwater at varying positions within clouds and in summer months, when the clouds are normally higher, the samples may be taken at the cloud base causing the apparent seasonal maximum.
Figure 6.3: TCAA concentrations in cloud and rain at two sites in Glentress Forest
The rain TCAA concentrations produced a more even temporal pattern with no maxima. The peak concentrations were found in Oct 98, Nov 98-Jan 99 (DHR) and in Nov 98, Jun 99, Aug 99 and Nov 99 (VR). Peak concentrations are not confined to summer months; some of the peaks occur in winter.

The pattern of precipitation volume is an important point to consider at the Glentress Forest sampling site. Unlike some Central European sites the rainfall pattern is not as simple as low volume in summer and higher volume in winter. If the monthly precipitation volume over the sampling period is considered (Figures 6.4, 6.5 & 6.6) it can be seen that different patterns exist for rain and cloud. The cloud deposition shows a seasonal pattern where the volume of cloud sampled is at a maximum in winter i.e. Feb 99 and Jan 00 with a gradual decrease to a minimum in summer i.e. July 99. This may help to explain some of the summer maxima found in cloud as well as the influence of cloud base with a fixed sampling point. Rainfall does not exhibit such a seasonal pattern, and generally both DHR and VR show a more consistent monthly volume throughout the year. Though peak volumes often occur in the winter i.e. Dec 99, and minima in summer i.e. Aug 99, some of the lowest rainfall may also occur in winter i.e. Feb 99.

Figure 6.4; Monthly wet deposition pattern in Dunslair Heights rain
Figure 6.5; Monthly wet deposition pattern in Dunslair Heights cloud

<table>
<thead>
<tr>
<th>Sample type/site</th>
<th>Conc. Range (ppb)</th>
<th>Mean conc. (ppb)</th>
<th>Median conc. (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHR</td>
<td>0.13 – 2.34</td>
<td>0.93</td>
<td>0.87</td>
</tr>
<tr>
<td>DHC</td>
<td>0.25 – 7.21</td>
<td>1.25</td>
<td>0.94</td>
</tr>
<tr>
<td>VR</td>
<td>0.15 – 1.55</td>
<td>0.72</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Table 6.10; TCAA concentrations measured at Glentress Forest

Figure 6.6; Monthly wet deposition pattern in Venlaw rain
The mean, median and range of TCAA concentrations determined at Glentress Forest are shown in Table 6.10. Generally the concentrations are in the same range as those found in the literature (c.f. Table 6.1), but show closer agreement with the results of Plümacher (1995), Schleyer et al. (1996) and Schöler (1991) at urban sites such as Berlin, Bonn and Hessen, than those determined at rural sites in Austria and Switzerland. This is unexpected as literature measurements (Table 6.1) suggested that urban pollution contains higher concentrations of TCAA in rain, so a rural site in Scotland experiencing ‘clean’ air masses might have been expected to have relatively low levels of TCAA. The concentrations measured at Glentress Forest are similar to those in large cities of Europe, suggesting TCAA might be formed from more globally averaged background chlorinated solvent concentrations. However, if the rain experienced at Dunslair Heights tended to be the first precipitation from an air mass for a long time, then this may explain why heightened concentrations were measured.

6.4.1.2 Rainfall weighted average concentration trend

A better representation of the TCAA concentrations determined at Glentress Forest is the rainfall weighted monthly average concentration. This allows the volume of precipitation to be corrected for, and smooths the seasonal pattern because the effect of small rain events, which may be very concentrated in pollutants, is diminished. The results are calculated as rain weighted means for 4 weeks (forward) and are calculated using Equation 6.2. The results are shown in Figure 6.7, 6.8 & 6.9. Each point represents a precipitation sample analysed in triplicate.

\[
\text{Rainfall weighted mean} = \frac{\left( \sum c_i \cdot v_i \right)}{\sum v_i} \quad \text{Equation 6.2}
\]

where; \( c_i = \text{concentration of TCAA} \)

\( v_i = \text{volume of precipitation} \)

\( \sum = \text{summation over next 4 week period} \)
Figure 6.7; Rainfall weighted monthly average TCAA concentration in Dunslair Heights rain (June 1998 to April 2000)

Figure 6.8; Volume weighted monthly average TCAA concentration in Dunslair Heights cloud (July 1998 to April 2000)
Figure 6.9; Rainfall weighted monthly average TCAA concentration in Venlaw rain (July 1998 to April 2000)

By looking at the individual volume corrected concentration the patterns shown in the graphs are much easier to distinguish. Single large points do not influence the graph as greatly as they do on the uncorrected concentration trends, as they are averaged over a month. There are several large concentration periods for DHR with maxima in July, September and November 1998 and also in August and December 1999, however the overall pattern remains fairly constant over the two year period (Figure 6.7). It is also interesting to note that there are very few low concentrations and none in the 0.1 ppb range which have been found as the average concentration in other sampling programmes. The cloud concentrations are still dominated by the high concentration event in May 1999, with other peaks in March 1999 and March 2000. The ratio of cloud : rain TCAA concentrations is not as great as with the raw data, but still up to 2.5. It is apparent from Figure 6.7 & 6.8 that there are periods such as August to December 1998 when the concentrations in rain exceed those in cloud.

Rain concentrations at Venlaw (Figure 6.9) show a very clear cyclical pattern. Though they follow the peaks experienced at Dunsclair Heights there is additional
fine detail. It is not known what causes this clear pattern of peaks and troughs. The concentrations are generally slightly lower at Venlaw than at Dunslair Heights. This can probably be explained by the seeder-feeder mechanism (see Figure 6.10) that operates in mountainous terrain, as discussed by Browning et al. (1974). An increase in the concentration of major ions at high altitude occurs when the cap cloud (feeder) droplets contain larger concentrations than the rain from higher levels. In this process, air containing aerosol is lifted by the hills and activated into orographic cloud with droplet radius typically in the range 3 to 15 μm. The cloud droplets in the orographic (feeder) cloud are efficiently scavenged by precipitation falling from higher levels, whereas the unactivated aerosol upwind of the high ground is mainly sub-micron and is not scavenged efficiently by falling rain. Measurements by Fowler et al. (1988) at Great Dun Fell of major ion concentrations in rain at the summit were a factor of 2.5 larger than in rain upwind of the hill. Though the difference is not as great at Glentress Forest this would explain some of the difference in concentration observed.

**Figure 6.10:** Seeder-feeder mechanism for enhanced rainfall concentrations of major ions in rain
The effect of using rainfall averaged monthly concentrations can be seen by comparing Table 6.10 & 6.11. The concentration range is made narrower, without affecting the mean or median concentration. The maximum rainfall weighted concentration for DHC (2.94 ppb) in Table 6.11 is much reduced compared to Table 6.10 (7.21 ppb). The rainfall weighting has made the concentration range, mean and median in Table 6.11 more similar for both DHR and DHC. It appears that some of the higher concentrations found in Central Europe such as 210 ppb by Juuti et al. (1997), which may lead to damage to tree health have not been measured in Glentress Forest. This suggests that damage is more likely to occur due to the long-term accumulation of TCAA in trees rather than a direct effect on foliage.

<table>
<thead>
<tr>
<th>Sample type/site</th>
<th>Conc. Range (ppb)</th>
<th>Mean conc. (ppb)</th>
<th>Median conc. (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHR</td>
<td>0.34 - 2.31</td>
<td>0.94</td>
<td>0.87</td>
</tr>
<tr>
<td>DHC</td>
<td>0.43 - 2.94</td>
<td>1.09</td>
<td>0.90</td>
</tr>
<tr>
<td>VR</td>
<td>0.25 - 1.31</td>
<td>0.71</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Table 6.11: Volume weighted monthly average TCAA concentrations at Glentress Forest

6.4.1.3 Wet input of TCAA to Glentress Forest

Another important parameter that can be calculated with these precipitation data is the input of TCAA to the forest over a specified time period. The input is calculated by multiplying the deposition in rain (millimetres per month) by the rainfall-weighted average monthly TCAA concentration (parts per billion) to give total input in µg m⁻² summed over 1 month. An estimate of direct cloudwater deposition to the forest must also be made, which includes the capture efficiency of the tree canopy. This is outlined in Equation 6.3. The input data are presented in Figure 6.11, 6.12 & 6.13 as monthly inputs of TCAA at the individual sites.

\[
\text{Input}_{\text{TCAA}} = v \times \mu \times \left( \frac{C_{d_{\text{forest}}}}{C_{d_{\text{gauge}}}} \right)
\]

\text{Equation 6.3}

\text{where;}
\text{Input} = \text{Input of TCAA to forest by cloud (µg m}^{-2}\text{), } v = \text{cloud deposition (mm) from the cloud gauge, } \mu = \text{rainfall weighted TCAA concentration (ppb), } C_{d_{\text{forest}}} = \]
The TCAA inputs show different patterns at the different sites. There was no great seasonal variation in rain input of TCAA at Dunslair Heights (Figure 6.11). The mean monthly input of TCAA in rain was 83 µg m$^{-2}$ over the whole period. There was significantly above average input in August, October and November 1998 and September 1999 and January 2000 whereas October and November 1999 and February and March 2000 had the smallest input of TCAA. These maxima and minima in TCAA input were in close agreement with the months of highest and lowest deposition respectively (Figure 6.4). With these exceptions the input was generally similar throughout the year which broadly reflects the pattern of wet deposition at Dunslair Heights. Both the concentration of TCAA and the volume of rain at Dunslair Heights only varies slightly through the year, so these findings are not surprising. The relatively uniform inputs of TCAA suggest that the patterns seen in the needle TCAA concentrations (Chapter 4) cannot be explained by variations in wet input at this site.

![Figure 6.11; Monthly TCAA input to Dunslair Heights by rain](image)

The input from cloud to forest at Dunslair Heights (Figure 6.12) shows an increase from a low level in summer 1998 to a maximum input in February 1999. From this
peak the input steadily decreases to a minimum period in summer and autumn 1999 and then gradually increases to another maximum in February 2000. This is clear evidence for a seasonal variation in TCAA input from cloud, related to the seasonal variation in cloudwater deposition. Crossley et al. (1992) suggested that for other major anions at Glentress Forest the input from cloud makes the greatest contribution to the total deposited despite the fact that cloudwater deposition only represents 25% of the hydrological input. The same phenomenon has not been found with TCAA because the magnitude of the input from cloud is less. The input is in the order of 8 times smaller from cloud with typically 11 µg m\(^{-2}\) per month compared to 83 µg m\(^{-2}\) per month for rain. This means that the total input of TCAA to the forest is not as sensitive to these minor seasonal variations in cloud input and is less likely to be detected in other matrices such as needles. Cloud is only responsible for 12% of input of TCAA in wet deposition at Dunslair Heights which is small compared to estimates by Crossley et al. (1992) that between 66 and 80% of the annual input of pollutants is by cloud. It is uncertain why a simple anion like TCAA does not act in a similar way to many other ions. One reason could be the origin of TCAA in the atmosphere. The enhanced concentrations of ions in orographic cloud is because droplets scavenge pollutants from the lower part of the atmosphere, largely from pollution sources close to the ground. Although this process may occur for TCAA, the evidence points to most of the TCAA being present in the air mass in which the rain forms, i.e. higher in the atmosphere, and more representative of the well-mixed lower troposphere.
The pattern of input from rain at Venlaw (Figure 6.13) is similar to Dunslair Heights. There are months with larger TCAA inputs, such as August 1998, and months with very small input, such as September 1998, but generally there is a uniform pattern throughout the year. This information is also important in understanding the cycling of TCAA. It was assumed that the lower site would have significantly different input of TCAA than at Dunslair Heights for 2 reasons;

(i) the seeder-feeder mechanism
(ii) direct cloud capture of TCAA

As discussed above the deposition of TCAA from cloud is small, so the wet input of 83 μg m\(^{-2}\) per month by rain at Dunslair Heights is only 50% greater than the input by rain at Venlaw of 57 μg m\(^{-2}\) per month. This suggests another reason why the concentrations of TCAA in needles (Chapter 4) are not as different at the 2 sites as may have been expected. This difference in TCAA input cannot be explained simply by the input of water to the two sites as there was only 12% more rain at Dunslair Heights over the study period than at Venlaw (Table 6.12).
6.4.1.4 Correlation of TCAA concentrations with meteorological properties

The only significant correlation between TCAA concentration and sample volume was for cloud at Dunsalar Heights (Figure 6.14). This shows a negative correlation between TCAA concentration and volume of cloud sampled with a significant relationship ($r^2 = 0.308; n=75$). There are two suggestions for the processes occurring. Firstly TCAA may be attracted to or formed on cloud condensation nuclei, from which clouds are created. As water is continually taken up by the cloud the concentration of TCAA is diluted. This would mean that the concentration of TCAA is proportional to 1/volume. A plot of this relationship is shown in Figure 6.15. The relationship is more significant than in Figure 6.14 and would tentatively indicate that TCAA in cloud is formed on cloud condensation nuclei before dilution. The second suggestion is that scavenging of TCAA may occur during storm events. Large precipitation volumes or long storm durations mean that TCAA is removed.

### Table 6.12; TCAA input and wet deposition by cloud and rain to sites in Glentress Forest (April 1999 to April 2000)

<table>
<thead>
<tr>
<th></th>
<th>DHR</th>
<th>DHC</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual TCAA input ($\mu$g m$^{-2}$)</td>
<td>891</td>
<td>125</td>
<td>669</td>
</tr>
<tr>
<td>Annual deposition (mm)</td>
<td>1062</td>
<td>118</td>
<td>956</td>
</tr>
</tbody>
</table>
This may lead to lower average concentrations in precipitation. This relationship is not necessarily linear and is dependent on frequency and duration of rain events.

Figure 6.14; Correlation between volume of cloudwater and TCAA concentration at Dunslair Heights

Figure 6.15; Correlation between 1/ volume of cloudwater and TCAA concentration at Dunslair Heights
6.4.2 TCAA in air

6.4.2.1 Field sampling results

6.4.2.1.1 Urban air sampling in Edinburgh

The temporal pattern of TCAA in air over the period February 1999 to April 2000 for Edinburgh is shown in Figure 6.16. The results are expressed in pg $\text{TCAA m}^{-3}$ and the error bars are standard deviations for the duplicate samples (if available).

Concentrations greater than 200 pg m$^{-3}$ were uncommon, with a mean concentration of 96 pg m$^{-3}$ for gas-phase measurements. There appears to be a slight seasonal variation in air TCAA concentrations, with the maximum values occurring in August and a period of not detectable concentrations in February. After close to zero concentrations in winter (Jan-March 2000) there was a steady increase in TCAA in air during spring. To establish if the TCAA concentrations had increased further during summer, measurements of TCAA were performed during August 2000 (Table 6.13). It should be noted that filter 1B from 19/8/00 had some contribution from particulate due to a leak so was probably overestimating TCAA, but the other 3 readings estimate a gas-phase concentration of 20 – 33 pg m$^{-3}$ over a 4 week period.
in August which was lower than expected. This demonstrates again that no summer maximum in concentration was observed and that peaks in atmospheric TCAA concentration are not associated with any particular time of the year. It should be noted that meteorological data from the Royal Observatory, Edinburgh, show that on average over 95 years the wettest month is August. Therefore it is no surprise that the gas-phase concentrations of TCAA in Edinburgh are low at that time of year.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Trichloroacetic acid in air (pg m(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 F</td>
</tr>
<tr>
<td>13/8/00 (116 m(^3))</td>
<td>9</td>
</tr>
<tr>
<td>19/8/00 (357 m(^3))</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 6.13; Gas phase and particulate-bound TCAA in air

The contribution of particulate-bound TCAA to air concentrations was much smaller than gas phase TCAA with most concentrations less than 50 pg m\(^{-3}\). The majority of the determinations of TCAA in particulate have given non detectable levels. As previously discussed it is possible that the PTFE filters are not efficient at collecting particles and so the particulate TCAA contribution is underestimated. However, in some cases particulate TCAA concentrations were at the same level as gas phase TCAA. It has been suggested that the majority of TCAA in air would be particle-bound and that gas phase TCAA would not exist due to its high solubility (Hoekstra et al. 1999a). This work has clearly demonstrated the presence of gas phase TCAA, but its effect on forest ecosystems is likely to be small due to its very low concentrations. The presence of TCAA as a gas phase entity is evidence for the formation of TCAA in the gas phase prior to dissolution into the aqueous phase, since it is unlikely that TCAA could be re-volatilised from solution. However, the lack of seasonal variation of TCAA in air does not support the hypothesis that TCAA formation is from photolysis of C\(_2\)-chlorinated solvents. A possible extension of this work would be to carry out a complementary sampling programme of TCAA in particulate using pre-weighed filters. Further work should attempt to accurately quantify concentrations and sources of particulate TCAA, such as power generation,
incineration and transport. Hoekstra et al. (1999a) suggested that according to the 'trace chemistries of fire' theory (Bumb et al. 1980) all types of oxidised chlorinated organic compounds can be formed in the presence of chlorine during combustion processes, which may then become adsorbed to particulate material. These may be major sources of TCAA in the atmosphere but so far they have not been greatly considered. There is only a small set of measurements of the flue gases of an incinerator (Mower et al. 1987), which gave TCAA concentrations in the range 370–3700 ng m$^{-3}$, three orders of magnitude higher than the ambient air results reported by Frank et al. (1995) and much higher than the maximum concentration (400 pg m$^{-3}$) reported during the Glentress Forest and Edinburgh monitoring programme. This is clearly a potentially significant source of TCAA that should be monitored more widely.

6.4.2.1.2 Rural air sampling at Dunslair Heights

The temporal pattern of TCAA in air over the period February 1999 to April 2000 for Dunslair Heights is shown in Figure 6.17. The results are expressed in pg TCAA m$^{-3}$ air and the error bars are standard deviations for the duplicate samples (if available).
The first important point to note is that the study at Dunslair Heights did not differentiate between the contributions from the gas and particulate phase to the total TCAA concentration. However, the total air concentration was nearly always smaller than the gas-phase concentrations at the urban site, with a yearly mean at Dunslair Heights of 30 pg m\(^{-3}\) compared to 100 pg m\(^{-3}\) at the urban site. This may be caused by differences in meteorology, with higher rainfall, cloud cover or humidity at Dunslair Heights due to the presence of cap cloud, or otherwise due to local sources of TCAA in urban areas. The possibility of a greater capture efficiency of particulate by the glass filters than for the PTFE filters used in Edinburgh would also have predicted a higher TCAA concentration. However, if as found in Edinburgh the particulate contains very low TCAA concentrations then this may not be important. The origin of particulate at Dunslair Heights is likely to be natural, especially sea salt and wind borne biological particles, so may have less TCAA associated with it.

6.4.2.2 Discussion

The variation in air TCAA concentrations at the 2 sampling sites challenges the belief that TCAA is formed from well mixed atmospheric chlorinated solvent concentrations, because if this were true the same air concentration would be expected in urban and rural areas. The real differences observed here may suggests an additional source of TCAA in the city, for example from chlorinated solvents in dry cleaning or from combustion processes. It is thought that these precursors are quickly dispersed and become well mixed in the atmosphere leading to an average background concentration. However, if comparing any two sites, one rural and one urban the comparison is more valid if sites with similar meteorological conditions are used, particularly including features such as orientation, rainfall and altitude, which may affect the concentrations in the gas-phase.

With only a few exceptions the peak air concentrations are found during the summer and the minima during winter, but the overall seasonal variation in concentration is small. The input of TCAA from the air is relatively constant throughout the year, and though small, may be significant to needle TCAA concentrations. If gas phase TCAA is deposited to either wet external surfaces of trees or internal needle spaces it
is likely to be dissolved, but it is not known if this will be taken into the core of the needles or subsequently leached out, as implied by the high TCAA concentrations measured in throughfall (Schleyer et al. 1996). It is possible that the constant uptake of air by trees through the stomata contributes in a small way to needle TCAA which is perhaps the most likely direct uptake of TCAA by needles.

It was assumed that due to the high solubility of TCAA there would be a negative correlation between volume of precipitation and air concentrations during a sampling period. Using the data from the meteorological sampling station at Dunslair Heights this was investigated. No significant correlation existed between the TCAA concentrations in air and any of the precipitation volumes measured.

The dry deposition of TCAA to forest can be estimated by assuming a deposition velocity of 1 cm s\(^{-1}\). This may be smaller if there is mostly particulate and larger if there is more gas phase TCAA, but is appropriate for the measurements that have been made in Scotland. The yearly input of TCAA to forest has been calculated by multiplying the deposition velocity by the mean air concentration at Dunslair Heights (30 pg m\(^{-3}\)), and is compared with TCAA input to forest by wet deposition at Dunslair Heights (Table 6.14). The input of TCAA by air is 10-fold smaller than cloud input and 2 orders of magnitude smaller than the total wet input of TCAA. This demonstrates that though the study of TCAA in air yields information about the formation processes of TCAA, the input of TCAA from air is insignificant compared to wet input.

<table>
<thead>
<tr>
<th>TCAA input (μg m(^{-2}) yr(^{-1}))</th>
<th>Dunslair Heights (rain)</th>
<th>Dunslair Heights (cloud)</th>
<th>Dunslair Heights (air)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>891</td>
<td>125</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 6.14; Annual input of TCAA to forest at Dunslair Heights by wet deposition

6.5 Conclusions

This chapter has described the first measurements in Great Britain of TCAA in both the aqueous and gaseous phase. The temporal trends in TCAA concentrations in rain
and, for the first time in cloud and air, have been performed at a remote, forested site. The data have not shown a seasonal variation with a maximum in the summer months, as described in the literature by several authors. The concentration range, median and mean TCAA concentrations in rain, as shown in Table 6.10, are in good agreement with those reported for several European cities such as Bonn and Berlin. However, the concentrations are much higher than reported for many similar rural sites in Austria and Switzerland. The origin of the larger rural TCAA concentrations measured in Great Britain is not known. It has been suggested that TCAA might be deposited on Great Britain before the prevailing south-westerly air stream proceeds towards Europe, consequently more depleted in TCAA. If the concentration measured in rain could be compared with the direction of approach of the air mass using back trajectories then maybe a relationship between westerly and easterly winds could be established. If easterly air masses have travelled across Europe dropping precipitation before arriving in Great Britain then these inputs might be depleted relative to those measured coming from an Atlantic direction. It is known that westerlies are wetter than easterlies and as such they may also contain more TCAA derived from the hemispheric background concentration.

The weekly-average concentrations of TCAA in cloudwater are enhanced compared with simultaneous rain concentration measurements, by as much as 7 times. The volume corrected average monthly concentrations have shown 2 to 3 times enhancement of concentrations in cloud. The overall input of TCAA to Glentress Forest in cloud has been shown to be small compared to rain, with only 10% of the total input of TCAA from cloud. This is greatly in contrast to the measurements of inputs at the same site for other anions such as chloride and sulphate. The input from cloud to forest of the other major anions was found to be 2 to 4 times greater than by rainfall despite the capture of cloudwater only representing 25% of the total wet input. A significant negative correlation has been found between the volume of cloudwater and the concentration of TCAA suggesting that dilution of TCAA occurs as clouds pick up more water vapour. The rain TCAA concentrations at Dunslair Heights (elevation 602 m) have been found to be higher than those at Venlaw (elevation 325 m) over the period April 1999 to April 2000. This has been explained
by orographic enhancement of rainfall by the seeder-feeder mechanism that operates in mountainous regions. This means that TCAA from the lower cap cloud is captured by rainfall from the feeder cloud at Dunslair Heights before being deposited on the forest. This is important because, as mentioned above, the cap cloud has significantly higher TCAA concentrations.

Air and particulate concentrations have been measured over a year at weekly intervals for the first time. Measurements have been made at both a rural, forested site and an urban site. The concentrations measured at the rural site have been found to be consistently lower than the urban site by a factor of 2 suggesting the existence of an urban source or formation process, though differences in meteorology may be responsible. The sampling methodology at the urban site has enabled some differentiation between gaseous and particulate-bound TCAA. The assumption that most TCAA is bound to particles due to its ionic form has proved untrue, with the majority of TCAA present in the gas-phase. However, it should be noted that the sampling method was not designed to efficiently trap all particulate, so the contribution from particulate matter may be underestimated despite the confidence in gas-phase results. A further programme of particle-bound TCAA measurements in air has been suggested to accurately estimate the particulate present and the amount of TCAA adsorbed on it, which may possibly originate from power generation, transport and incineration.

The data produced from air, cloud and rain measurements are useful for the mass balance calculations of the Glentress Forest ecosystem (see Chapter 8). The annual input of 1030 µg m⁻² TCAA measured at Glentress Forest from combined wet and dry deposition is 30% higher than that calculated by Hoekstra et al. (1999a) for spruce forests under Dutch and German environmental conditions (700 µg m⁻²). It should be noted that no contribution was included for the cloud input to Dutch and German sites, which may make the estimates more closely agree. It is likely that cloud input of TCAA is also important in upland German locations.
Chapter 7

Long term exposure of Sitka Spruce seedlings to trichloroacetic acid: the effects on needle and soil concentrations and tree detoxification mechanisms

7.1 Introduction

The measurements presented in previous chapters have shown that trichloroacetic acid is present in varying concentrations in needles, soil, precipitation and air in a UK forest. A discussion of the sources of TCAA in the natural environment has been made and evidence for natural formation has been presented. As yet no evidence has been presented as to the mechanism and the route of uptake of TCAA from these compartments which lead to the measured concentrations present in Sitka spruce needles. Much of the toxicology and uptake work in the literature was performed as a consequence of the use of TCAA as a herbicide and therefore large concentrations were used to determine its efficacy and mode of action. The current situation, in which there is no longer a direct source of TCAA from use as a herbicide and the environmental exposure from atmospheric input is at a trace level, poses different challenges, and so earlier work on its uptake and effects at high concentrations should be considered cautiously. As a result of this, the relevant research in this area is sparse and many questions remain to be answered.

The physical and chemical properties of TCAA determine the pathways by which TCAA may enter trees. The high water solubility and low lipophilicity of TCAA means that TCAA is likely to exist predominantly in the aqueous phase and is unlikely to penetrate the highly protective lipid surfaces of coniferous needles on contact. Its low vapour pressure and high Henry’s Law constant mean volatilisation from the soil or water courses is not likely and so exposure to trees is thought to occur via the roots. Uptake is influenced by other environmental conditions such as temperature, moisture and organic matter content of the soil, and also the plant species of interest.
Although root uptake is the most likely pathway, theories exist to explain how TCAA may be taken up through the hydrophobic cuticle of trees. Burkhardt & Eiden (1994) suggested evidence for the existence of thin water films on the surface of needles which could act as bridge or liquid junction between the interior and exterior of the needle without blocking the whole stoma. It was suggested that these water films led to the dissolution of trace gases and the migration of ions along the needle surface. If the concentration of anions build up on the needles by continuous wetting and drying cycles then this would lead to an uptake of these anions. This situation is more realistic the closer the particles are to the stomata, as suggested by Jagels (1991), who reported that fog droplets are preferentially deposited near the stomata. If this transfer of anions across stomata holds true for trichloroacetate then the importance of wet input from cloud cannot be underestimated. The concentrations of many common anions in cloudwater have been reported to be enriched compared with that in rainwater at the same site (Crossley et al. 1992, Sigg et al. 1987), which makes this possible foliar input route even more important.

The role soil plays in the uptake of TCAA by trees is still uncertain, but without doubt the uptake of precipitation via a root pathway through the soil is a major contributor. Whether the soil accumulates, enhances or indeed breaks down TCAA from precipitation is a moot point. The ability of roots to take up TCAA from soil solution is not in doubt, but whether more active root processes enable the uptake of TCAA possibly bound to the soil is not known. It seems that the concentration of TCAA in soil solution is the most important parameter. It has been found that TCAA is transported rapidly within plants once it has been taken up. Studies using barley and oats (Schroll et al. 1994) showed that TCAA could be transported from roots to shoots and vice versa. Other work by Uhlírová et al. (1996) using $^{14}$C-labelled TCAA on fir trees reported uptake by roots and rapid distribution within a few hours to needles and leaves. The common theme of much of this research is the use of very high concentrations of TCAA, which are not representative of true environmental concentrations. With this background knowledge it is clear that the TCAA concentrations expressed in the tree needles are representative of some contribution
from atmospheric deposition, soil concentrations and TCAA metabolism within the trees, though the extent of each is not known.

A further theory for the presence of TCAA in tree needles is via the direct uptake of chlorinated solvents such as perchloroethylene into the needles of trees. It was proposed by Frank et al. (1992) that after the uptake of these precursors transformation occurred either by photolysis or by enzymatic detoxification by P-450 monooxygenase. This hypothesis was tested by Juuti et al. (1996), who determined the needle TCAA concentrations in trees exposed to differing light intensities. No statistical differences were found between high and low light exposures suggesting TCAA in the needles was not formed by the in situ conversion of chlorinated precursors. It was also considered that the enzymatic detoxification process which normally only exists in the livers of humans and animals is unlikely to occur in trees. However, some evidence has been published suggesting that hybrid poplars can degrade trichloroethene to TCAA amongst other things (Newman et al. 1997). This still remains the most unlikely route of exposure and uptake of TCAA by trees, because even if TCAA is produced in the cuticle it is not known if this will eventually end up in the core of the needle matrix. Direct formation of TCAA within the needle core is not possible because most leaf epidermal cells contain pigments which prevent UV from penetrating the leaf.

To try and discern which are the more important uptake pathways, work has been carried out with seedling experiments particularly on Scots Pine (Pinus sylvestris L.). Long term experiments by Sutinen et al. (1995), which involved treatment via root and foliage for two simulated growing seasons showed that both routes led to increases in TCAA concentrations in needles. It was argued that the primary uptake route was via the roots and that most TCAA taken up by foliar treatment led to adsorption on the needle surfaces instead of actual uptake. However, this was shown to be important as it caused disintegration of epicuticular waxes and stomatal cells, an effect not seen after the root uptake. The high concentrations found in some of the sprayed trees were explained as anomalies and no real explanation was offered for the
TCAA concentrations from the sprayed trees [60-80 ng (g fwt $^{-1}$) TCAA] being higher than those of the watered trees [40-60 ng (g fwt $^{-1}$) TCAA] at the lower concentration application. It was also found that the current year needles gave between 1.5 and 4 times lower concentrations than C+1 needles in foliage and root applications. As mentioned in their discussion the penetration of TCAA through the lipophilic cuticle is improbable and entry via the stomata is unproven, despite the possibility of liquid junctions, even if sprayed during daylight hours. Therefore there appeared to be some uptake mechanism at work which was so far undefined. Further work by Sutinen et al. (1997) looked at the uptake and effects of exposure to two concentrations of TCAA and monochloroacetic acid (MCAA) on the trees, with a focus on the disruption of growth. In this study the exposure pathways were combined which meant that the effects caused by either application could no longer be distinguished. This too showed an increase of TCAA in needles in conjunction with increases in potassium and nitrogen concentrations of needles. The same trees showed dramatic changes in the activity of xenobiotic detoxification enzymes in the needles, notably peroxidase and glutathione-S-transferase (Schröder et al. 1997), which suggested metabolism of TCAA at low concentrations had occurred. The common feature of these excellently designed and managed dosing experiments was the use of considerably higher concentrations than found in the environment. For example, the concentrations of TCAA in mist used by Sutinen et al. (1995) was 1 and 50 mg l$^{-1}$, which is perhaps 10000 times higher than measured at Dunslair Heights (see Chapter 6). The total input of 23 and 43 μg TCAA to the soil during the experiment (Sutinen et al. 1997) represented the equivalent dosage of 100 and 200 μg m$^{-2}$, which was similar to the annual wet input of TCAA to Finnish forests (Juuti 1997). Whether it is the total dose or the concentration of the exposure that is more significant is not known, but the huge concentrations used for mist application may well explain the damage observed to wax structures and occluded stomata.

A further interesting aspect of this work is the physiological damage detected when trees are exposed to environmental concentrations of TCAA. It is well known that TCAA can induce major structural damage to needles if applied in large enough
concentrations (Ashton & Crafts 1973), but the effect of environmental concentrations in the low ppb range is less well documented. Highly eroded wax structures have been reported in Berlin and its surroundings (Plümacher 1995), and Frank et al. (1994) have positively correlated branch damage with needle TCAA concentrations. It is difficult to elucidate the possible synergistic effects of multiple air pollutants in the field so the study of damage by direct experimentation is the most revealing methodology. Schröder et al. (1997) demonstrated a change in the activity of xenobiotic detoxification enzymes in response to the addition of TCAA to trees. The peroxidases (POX) are ubiquitous in plants and are principally enzymes that oxidise xenobiotics in plants with a wide substrate specificity. The activity of POX in roots and leaf tissue has been suggested as an indicator of pollution (Castillo et al. 1987; Markkola et al. 1990) and environmental stress (Castillo 1986). Glutathione-S-transferase (GST) has been found to catalyse the conjugation of glutathione with a number of pesticides and herbicides in plants (Lamoureux & Rusness 1989 & 1993; Sandermann 1992 & 1994). The role of GST in xenobiotic metabolism is well known (Hunaiti & Ali 1990; Dean et al. 1990) and its induction has been observed in response to volatile chlorinated hydrocarbons (Schröder et al. 1997). POX and GST activity have been measured in growing experiments to demonstrate a detoxification response in trees to TCAA. The required reaction chain consists of three distinct phases (Coupland 1991):

Phase I: Oxidation, Reduction, Hydrolysis

Phase II: Conjugation (sugars; Glutathione)

Phase III: Metabolism/ Transport

Whilst phase I reactions might lead to the formation of toxic intermediates, plants do also possess detoxification enzymes e.g. GSTs. These enzymes are able to form water-soluble compounds from xenobiotics by conjugation with the tripeptide glutathione. Conjugates like this can be transported in the plant and are subject to rapid metabolism.
7.2 Experimental aims

This chapter presents the results of similar experiments on Sitka Spruce seedlings [\textit{Picea sitchensis} (Bong.) Carr] over a growing season. Trees were exposed to two levels of TCAA via two separate exposure pathways (foliage only and soil only) over the period May 1999 to October 1999 in a greenhouse environment. The aim was to investigate:

- whether one or both of the exposure routes resulted in increased needle TCAA concentrations using, wherever possible, environmental TCAA concentrations, and
- the observation of any physiological or biochemical effects.

The concentrations of TCAA in the soil were also investigated. The trees were left over the winter period without TCAA applications in order to investigate how the needle concentrations decreased over time, a period when the tree metabolism had effectively stopped. Needle and fine root samples were analysed for protein content and GST activity to investigate if detoxification mechanisms were affected by treatment with TCAA.

7.3 Experimental

7.3.1 Seedling material

Two year old potted seedlings of Sitka Spruce [\textit{Picea sitchensis} (Bong.) Carr] of Queen Charlotte Island provenance grown at the Centre for Ecology and Hydrology, Bush Estate, Scotland were used in the experiment. The seedlings were grown and treated according to normal nursery practice, including fertilisation and occasional pesticide treatment. The original 147 seedlings available were re-potted into 20 cm pots using a peat/loam/quartzage-grit mixture (3:1:1 v/v). After several weeks growing outside the trees were graded in order of size. From the 147 trees, 27 of the tallest and smallest seedlings were rejected leaving 120 trees for the experiment. These trees were immediately moved inside to an unheated glasshouse. The glasshouse was fitted with a fan, which was activated if the temperature rose above 25°C. During the experiment the pots were placed in plastic saucers and the trees
were irrigated by filling the saucers with tap water rather than direct irrigation to the soil. This stopped any applied TCAA being leached out of the soil. The irrigation frequency was 3 times a week at most during the summer period and was enough to maintain healthy trees. Irrigation was applied equally to all trees.

7.3.2 Experimental design
There were 6 individual combinations consisting of 3 TCAA application levels (L0, L1, L2) and 2 types of treatments (T1 and T2). The 120 trees were divided into six groups, starting with the tallest working down to the smallest. Each group possessed one of the six tallest, one of the second six tallest and so on until each group had one of the six smallest trees. These groups of 20 were themselves split into 4 batches of 5 trees with Batch 1 (B1) in all treatments containing the tallest trees and Batch 4 (B4) containing the smallest trees. Therefore in all groups of trees there was a height gradient from B1 to B4. All of the trees were labelled on the pots and on the stems, and for easy recognition each had a coloured tag pertaining to the specific treatment i.e. L_nT_m, Batch x. The pots were placed in saucers and the batches of trees were randomly arranged in the glasshouse. The trees were rearranged twice throughout the treatment period to account for any bias or favourable conditions due to the positioning of the batches within the glasshouse. The individual trees within each batch were rearranged every time an application was performed. All of the trees were fitted with a protective plate around the stems at soil level, which overhung the edge of the pots, to avoid any drip from the spray treatments contacting the soil. All trees (even soil applications) had a plate fitted in case it affected the growing conditions in any way. Once brought into the glasshouse at the start of the experiment the initial height and root collar diameter of all the trees were measured.

7.3.3 Exposure technique
The seedlings were exposed to TCAA during the period May 7th 1999 to October 8th 1999 which included the full tree growing season. The treatments were performed on average every 3 days which totalled 47 applications during the above period.
The TCAA solutions were applied via the soil only (T2) and the foliage only (T1) using three treatment concentrations: low (L1), high (L2) and control (L0). The TCAA solutions were prepared by diluting a stock standard of approximately 2.5 ppm TCAA with deionised water to give 10 ppb and 100 ppb solutions of TCAA. The control solution was the same deionised water used to prepare the standard solutions. For the soil applications (T2) approximately 63 ml of the above solutions were added directly to the pots. For the foliage applications the seedlings were removed from their saucers and batch by batch moved to the spraying area. Here each batch of trees were sprayed evenly with 315 ml (5 x 63 ml) of the above solutions and returned to their positions ensuring any leftover solution on the protective plates was removed. A hand sprayer was used to generate the fine mist, but this did not allow a determination of the droplet size. The order of spraying was always L0, L1 then L2, which meant that any residue left on the floor of the spraying area was insignificant compared to the applied treatment dose. The volume of 63 ml per tree was selected as this represented 2 mm of rain for the size of the seedlings used, which was the approximate threshold for needle drip to occur.

7.3.4 Determination of TCAA concentration in needles

Needles were sampled on 3 dates: August 1999 (preliminary), Oct 1999 (1st sample) and May 2000 (final sample). At each date a current year shoot (1999) was taken from each tree of the batch to be analysed and thoroughly rinsed with deionised water and dried on blotting paper. The needles were removed by plunging the shoot into liquid nitrogen, pooled and stored in a freezer until analysis. Stem material of the current year needles was also sampled in October 1999 and analysed for TCAA.

The preparation and analysis of needles for TCAA followed the same procedure as outlined in Section 4.4.1 with the exception of the sample mass weighed. Due to the lack of needle material available a mass of 0.5 g (± 1mg) of sample was weighed into the 20 ml glass headspace vials previously heated and cooled to drive off any CHCl₃. After HSGC analysis the peak areas obtained were used to calculate the TCAA
present in the samples according to Equation 3.1 in Section 3.3 using the factor 1.26 determined in Table 3.5.

The procedure used for the determination of TCAA in stems was the same as for needles, but due to the lack of material only one determination for each treatment was possible by combining the stems of all four batches. Therefore results are reported for the six treatments i.e. L0T1, L2T1 etc.

For each needle sample prepared the dry weight: fresh weight ratio was determined as outlined in Section 4.4.2 to give a concentration in ng (g dwt)\(^{-1}\) TCAA.

### 7.3.5 Determination of TCAA in soil

A 10 cm soil core of each pot was sampled using a 2 cm diameter finger corer after the October 1999 harvest. The soil from the 5 replicate trees in each batch was combined, sieved and blended. The soil samples were then analysed as outlined in Section 5.4.1.

### 7.3.6 Determination of height and root collar

The height and root collar of the seedlings was determined three times. Firstly at the start of the experiment, then in October 1999 and finally in May 2000. A metre rule was used to measure the height between the rim of the pot and the tip of the highest point of the tree, which sometimes had to be manually straightened to give an appropriate result. The root collar was measured at the level of the pot rim using a set of digital callipers. Two measurements were taken for each tree to allow for imperfections in the stems. The average diameters were used to convert to a cross sectional area of the stem. The percentage increase in height and root collar during the experiment was determined.

### 7.3.7 Needle partitioning experiments

Laboratory based partitioning experiments were performed to attempt to mimic the processes that were occurring in the growing experiments. It had been suggested that
TCAA is not able to enter the needles from foliar application either through the stomata or the cuticle, so to test this hypothesis the following partitioning experiments were carried out.

Newly flushed needles from Venlaw in Glentress Forest were sampled (12/5/99) and manually removed from their stems so as not to damage their surface structure. After rinsing in water and drying on blotting paper approximately 3 g of needles were weighed into round bottom flasks (RBF). The experiment was designed with 3 treatment levels (L0, L1, L2) which corresponded to HPLC water, 10 ppb and 100 ppb TCAA solutions respectively. The experiment was conducted for 8 exposure times: 0, 15, 30, 45, 60, 120 minutes, 6 hours and 23 hours. At the start of the experiment the RBF was filled with the appropriate solution so that no air bubbles existed and was capped. After exposure the solution was removed and the needles were thoroughly rinsed and dried. The needles were then frozen until analysis.

At the time of analysis the needles were removed from the freezer and the analysis was performed as outlined above in Section 7.3.4. The TCAA concentration in each set of needles was determined and the efficiency of partitioning assessed.

The experiment was repeated using solutions of TCAA adjusted to pH 4. The reason for this adjustment was that by reducing the pH closer to the pKa of TCAA (pKa = 0.7) a higher proportion of TCAA should exist in the associated form rather than the ionised form, which may affect the uptake.

7.3.8 Isolation and measurement of Glutathione-S-transferase

Fine non-lignified parts of the roots and shoots of Year C needles were taken from each seedling for enzyme analysis at the main sampling date in October 1999. The samples were cut using a clean knife and rinsed with deionised water, dried carefully with tissue paper and stored at -80°C. The samples of needles and roots from the individual seedlings of each batch were combined to give a pooled batch sample. The
samples were sent on dry ice to Forschungszentrum für Umwelt und Gesundheit (GSF), Neuherberg, Germany for the determination of GST and POX activity.

GST activity was determined in the root and needle samples by Peter Schröder of GSF, according to the procedure described previously (Schröder et al. 1997). Needle and root samples were ground with liquid nitrogen to a powder, and 10 volumes (wt/v) of 100 mM Tris/ HCl buffer added at pH 7/8 containing 1% PVP K30, 5 mM EDTA and 0.25% Nonidet P40. The slurry was homogenised, allowed to stand and centrifuged and then the supernatant was filtered. The GST activity was determined with aliquots of this crude extract. Solid ammonium sulphate was added to these pooled extracts in two steps to 40% and then 80% saturation. After centrifugation, the pellet was re-suspended in 0.025M potassium phosphate buffer. Aliquots of the re-suspended samples were applied to an affinity chromatography coupled to a HPLC system. After application of the protein extract, the column was rinsed with potassium phosphate buffer until no elution of protein was observed at 254 nm and GST was eluted from the column with a linear gradient of reduced glutathione (Schröder & Berkau 1993). Each fraction of the HPLC runs was checked for GST activity using 1-chloro-2,4-dintrobenzene (CDNB) as a substrate, and the fractions containing GST activity were pooled for the determination of catalytic properties using CDNB and 1,2-dichloro-4-nitrobenzene (DCNB) as xenobiotic substrates. GST activity was determined spectrophotometrically using CDNB as a model substrate according to Habig et al. (1974) and with DCNB following the assay method of Schröder et al. (1990). Protein contents were determined by the method of Bradford (1976) using bovine serum albumin as a standard.

GST activity was measured in triplicate, but protein content only in duplicate. Incomplete analysis meant only the protein content and the GST activity with DCNB as a xenobiotic substrate was reported.
7.4 Results

7.4.1 Preliminary needle analysis

Figure 7.1 shows the preliminary results from the experiments sampled in August 1999. To test whether the dosing experiments had any effect on the needle TCAA concentrations, needles were sampled from both treatments at the highest TCAA concentration exposure (L2) and were compared with the needle concentrations in one of the control sets. LOT2 was chosen as the appropriate control as it was assumed that the addition of deionised water directly to the pots would show the maximum intrinsic TCAA to be found within the control trees. This was designed to show if the applied concentration of TCAA was high enough to give a noticeable effect and whether uptake had occurred via both exposure routes.

![Figure 7.1: TCAA concentrations in the needles of Sitka Spruce (August 1999)
(Each bar is a mean ± standard deviation of triplicate analyses of a pooled sample of each batch)](image)

The results in Figure 7.1 show that TCAA concentrations increased in needles exposed to the L2 treatments. There are several important points about the results that are key to the experiment. Firstly, the control trees show reproducible low TCAA concentrations, which is essential in order to demonstrate whether the effects
of the treatments are statistically significant. The concentration of TCAA in the tap water used to irrigate the trees was measured to be about 5 ppb and it was of concern that during the summer, when frequent irrigation was required to keep the trees healthy, the contribution due to watering might obliterate the response due to TCAA applications. The TCAA concentrations in the control trees were uniform within the four batches. This suggests that the protocol for filling the saucers with water is a precise way of irrigating all the trees equally. The single result for outside trees (trees from the original batch not used in the experiment and left outside the greenhouse) is shown to illustrate the difference in needle concentrations after irrigation with tap water and rainwater. The outside trees were rarely watered with tap water as the input from rain was sufficient. Irrigation by rain introduces less TCAA as the concentration of TCAA in rain is in the 0.1 ppb range compared with 5 ppb in the tap water. Note that a direct comparison is not totally appropriate as the trees growing outside are exposed to different temperature and UV light levels and are therefore different in hardiness and wax structure and so may respond differently to TCAA addition. These data suggest that some other source of irrigation should have been used in the experiment so as to minimise the blank/ background levels, but unlike the work by Sutinen et al. (1995 & 1997) that used nearby lake water for irrigation, there was no natural source of water convenient to the glasshouses. The second important feature of the experiment illustrated by the comparison between inside and outside trees is that the addition of increased TCAA to trees in tap water directly led to increased needle TCAA concentrations.

The general point to note about the L2 results is that the needle concentrations are all higher than the controls, whether TCAA is applied via T1 or T2. ANOVA of the results (not shown) gives highly significant differences between the control and both L2T1 and L2T2 (p<0.05). The concentrations in the L2 trees are more variable between batches than are the controls, but the between batch variance is not statistically significant. These findings suggest that both routes lead to TCAA being taken up. L2T2 shows that TCAA is taken up via the soil to produce TCAA concentrations in the range 130 to 210 ng (g dwt)^{−1} TCAA, but L2T1 shows that
TCAA may be taken up by foliar application to give concentrations in a similar range of 180 to 260 ng (g dwt)$^{-1}$ TCAA. This is surprising for two reasons. Firstly, an above ground uptake pathway has been demonstrated. Whether uptake occurs through the needles or some other route is not certain, but the measured concentrations are not residual TCAA on the surface because all the needle were rinsed prior to analysis. Secondly, not only is there uptake of TCAA via foliage, but the needle TCAA concentrations were similar to those produced from root uptake. Despite the attempt to supply a similar dose by foliar and root applications during the spraying of the trees the efficiency of interception/ capture of spray was low and so much of the applied dose was immediately lost. This suggests that the uptake of TCAA by foliage is more efficient than the soil route. One possible explanation is that on application to the soil, TCAA may become bound to the soil organic matter/ humic material and thus be unavailable for uptake. Alternatively TCAA may be degraded in the soil before it can be taken up. The higher variability within the L2 batches than in the controls suggests that local factors associated with foliage/ soil affect uptake efficiency. It is apparent that batch 2 of both L2T1 and L2T2 display the highest needle concentration within their groups which as yet cannot be explained.

The treatment continued until October 1999 when a full analysis was performed. Care was taken to minimise the irrigation with tap water so as to reduce the control levels in the experiments, which had been identified as a key factor from these preliminary findings.

### 7.4.2 Full analysis of needles (October 1999)

A full analysis of needles was performed in October 1999 after the trees had ceased growing for the season and the treatment programme had stopped. The needles were sampled 10 days after the final application and were stored frozen until analysis. All shoots were rinsed with deionised water to remove any residual surface TCAA. The TCAA concentrations were determined by HSGC analysis and are shown in Figure 7.2.
Comparison of Figure 7.1 & 7.2 shows that the initial pattern observed in August 1999 is still displayed in these results with added complexities due to the extra data. The needle TCAA concentrations in the October samples have approximately doubled compared to the August determinations, as expected after a further two months of TCAA applications. The general trend of the data is increasing TCAA concentrations in the needles of both treatments with increasing TCAA dose/ exposure.

![TCAA concentrations in needles of Sitka Spruce (October 1999)](image)

Figure 7.2; TCAA concentrations in needles of Sitka Spruce (October 1999)

*(Each bar is a mean ± standard deviation of triplicate analyses of a pooled sample of each batch)*

The outside trees still display the lowest TCAA concentrations, but have increased by a third since the preliminary sampling period from 30 to 40 ng (g dwt)^-1 TCAA. The control trees for both treatments show remarkably consistent needle TCAA concentrations. With the exception of LOT1 B3 and LOT2 B2 the remaining six control batches are very similar. It is possible that differences in the soil composition of the pots may have had an effect but this is unlikely as the soil was mixed as homogeneously as possible at the start of the experiment when the trees were repotted. Each bar represents the pooled value from 5 individual seedlings. A further point to consider is the pattern shown in LOT2 in August 1999. These results (Figure
7.1) showed that the concentration in LOT2 B2 was also slightly higher than the other batches in August. This would seem to point to increased water use in this batch relative to the others or some sort of contamination.

The results determined in this complete harvest of trees in October 1999 can be analysed statistically using a two-way Analysis of Variance (ANOVA) using batches as replicate blocks. The results of ANOVA for the needle TCAA concentrations are shown in Table 7.1. For this procedure the mean needle concentration for each batch was used to elucidate whether any significant differences existed between treatment type and between the applied concentration.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels</td>
<td>106119.27</td>
<td>2</td>
<td>53059.64</td>
<td>25.39</td>
<td>0.00</td>
<td>3.55</td>
</tr>
<tr>
<td>Treatments</td>
<td>3959.09</td>
<td>1</td>
<td>3959.09</td>
<td>1.89</td>
<td>0.19</td>
<td>4.41</td>
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<tr>
<td>Interaction</td>
<td>1037.68</td>
<td>2</td>
<td>518.84</td>
<td>0.25</td>
<td>0.78</td>
<td>3.55</td>
</tr>
<tr>
<td>Within</td>
<td>37610.12</td>
<td>18</td>
<td>2089.45</td>
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<td></td>
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<td>Total</td>
<td>148726.16</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 7.1; Two-way ANOVA of needle TCAA concentrations in October 1999 (all results)

Table 7.1 shows a significant difference in needle TCAA concentrations between the levels of application (P<0.001). This was the expected result as greater exposure to the trees was expected to lead to more uptake of TCAA. However, no significant difference exists between the two different treatment types. These results imply that, irrespective of whether the trees are sprayed or watered, a similar concentration of TCAA is found in the needles. As discussed above, this means that the route of uptake of TCAA by trees is both via foliage and roots and is not dominated by one pathway. However, it may mean that in a forest the route of uptake is determined by the mode of wet input present (cloud or rain).

The investigation of differences between batches within a treatment can be performed using two-way ANOVA without replication as shown in Table 7.2a & 7.2b. As batch
number is related to the height grading performed at the beginning of the experiment, variation in TCAA concentration due to tree size can be examined. In Table 7.2a the results are shown for treatment 1 only. The statistics show that there is a significant difference in needle TCAA concentration between the levels of application in treatment 1 (to foliage) (P=0.01), but no significant variation between the batches. This suggests that tree size does not affect the TCAA concentration in needles. A similar analysis of trees undergoing treatment 2 (to soil) yielded the same conclusions (Table 7.2b).

To assess the effect of treatment with low concentrations of TCAA the increases in TCAA concentration in the L1 trees are considered with respect to L0 controls and with respect to the treatment type. A valid conclusion to this work is that changes in needle concentration due to uptake of 10 ppb TCAA (L1) is detectable via both roots and foliage, but is not consistent through all the batches. This situation could be improved by striving for a lower blank/ control level as discussed above, which would improve the ‘detection limit’ of changes occurring in L1 trees.

When considering L2T1 trees it can be seen that with the exception of B3 all the trees exhibit higher needle TCAA concentrations than the controls. The uptake of TCAA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels</td>
<td>63384.64</td>
<td>2</td>
<td>31692.32</td>
<td>11.65</td>
<td>0.01</td>
<td>5.14</td>
</tr>
<tr>
<td>Batches</td>
<td>5846.14</td>
<td>3</td>
<td>1948.71</td>
<td>0.72</td>
<td>0.58</td>
<td>4.76</td>
</tr>
<tr>
<td>Error</td>
<td>16326.74</td>
<td>6</td>
<td>2721.12</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>85557.52</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.2a; Two-way ANOVA of needle concentrations for foliage treatment: T1

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels</td>
<td>43772.30</td>
<td>2</td>
<td>21886.15</td>
<td>12.80</td>
<td>0.01</td>
<td>5.14</td>
</tr>
<tr>
<td>Batches</td>
<td>5178.85</td>
<td>3</td>
<td>1726.28</td>
<td>1.01</td>
<td>0.45</td>
<td>4.76</td>
</tr>
<tr>
<td>Error</td>
<td>10258.40</td>
<td>6</td>
<td>1709.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59209.55</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.2b; Two-way ANOVA of needle concentrations for soil treatment: T2
via foliage after treatment with the higher concentration (100 ppb) produced a more consistent needle TCAA concentration of 260 to 360 ng (g dwt)$^{-1}$ TCAA. The interesting point is that these concentrations are not significantly higher than the maximum concentrations measured in L1T1 needles. This may point to the existence of a steady state within the needles maintained by a process of metabolism. A similar situation is encountered for L2T2 trees. This treatment produced a consistent TCAA concentration in the range 250 – 300 ng (g dwt)$^{-1}$ TCAA, which was generally lower than L2T1 trees. This points to a possibly more consistent uptake route/ pathway via foliage, but with broadly similar concentrations in both L2T1 trees and the maxima measured in L1T2 trees. This may again point to the existence of a steady state concentration maintained by metabolism processes, though the mechanism and the subsequent metabolites are not known. This experiment is not sophisticated enough to monitor and elucidate the mechanism of metabolism that is possibly occurring because of the few temporal sampling times. If a temporal study was performed it is possible that a large increase in concentration would be found after exposure followed by subsequent metabolism. A variation on this experiment would be to expose the trees with 1 large dose of TCAA and then monitor the changes in needle concentrations over time. Many other workers (Sutinen et al. 1995 & 1997; Matucha et al. 2000b) have rapidly found a peak in TCAA concentrations, but this is usually the result of an exposure to very high single TCAA applications and so may not be applicable to the study in this thesis. The only true way to elucidate the metabolic mechanisms would be to use labelled TCAA and to monitor the trees in a closed system. This is a far more complex experiment, especially given that Matucha et al. (2000b) have suggested that $^{14}$C-TCAA was metabolised to $^{14}$CO$_2$, which would have to be trapped and analysed.

The most striking result in this experiment is the existence of an equally important foliar uptake pathway, which had previously been assumed to be a minor contribution to the needle TCAA concentrations. It is widely believed that TCAA cannot pass directly through the waxy cuticle of Sitka spruce needles, though the existence of liquid junctions has been described (Burkhardt & Eiden 1994). The evidence for an
above ground uptake pathway is clear, so a hypothesis was put forward that the stems played a role in the uptake of TCAA. Investigation of this hypothesis involved the analysis of stem material from the shoots sampled on October 1999. The stems from the 4 batches of each treatment were combined to give enough material for the analysis of TCAA. Though it was only possible to determine an average TCAA concentration per treatment, this was enough to investigate differences between T1 and T2 shoots, which might indicate an uptake through stems. The stem TCAA concentrations determined in the six level-treatment combinations are shown in Figure 7.3.

The TCAA concentrations in the stem material from the foliage treated trees (T1) are higher than in the soil treated trees (T2) at all TCAA application levels. It is unclear why the L0T1 stems appear to give much higher concentrations than in any Li-treated stems. A single repeat preparation and analysis of L0T1 stems determined a lower concentration \([47.5 \text{ ng (g dwt)}^{-1} \text{TCAA}]\), which is a more appropriate background/ control level, is lower than the L1T1 stems and suggests that the concentration originally measured was an anomalous result. The pattern in Figure 7.3 illustrates increasing stem TCAA concentrations with exposure level by both treatment T1 and T2. The increase in stem TCAA concentration from L0T2 to L1T2 to L2T2 shows that the stems act as a conduit for soil solution taken up \(via\) the roots. As higher TCAA concentrations were applied to the pots the TCAA in the xylem increased, resulting in stem concentrations of 19, 26 and 69 ng (g dwt)\(^{-1}\) TCAA for L0, L1 and L2 respectively. However, it appears that the TCAA concentrations in the foliage treated stems (T1) does not closely resemble the concentrations expected if the stems were purely a conduit of its irrigation water \(via\) the transpiration stream (L0T1). There is higher stem TCAA concentrations with increasing spraying level, despite the fact that all T1 treated seedlings received identical TCAA from irrigation in the soil. The T1 stem concentrations were 61 and 277 ng (g dwt)\(^{-1}\) TCAA for L1T1 and L2T1 respectively. This is far higher than the comparable T2 values and higher than the controls, which suggests that TCAA input must also occur through the stem surface. There is a contrast between the large differences observed between
T1 and T2 stem concentrations compared to the broadly similar needle concentrations found in the T1 and T2 treatments at each level. Table 7.3 shows a comparison between the concentrations measured in the stems and the needles in October 1999.

Table 7.3; A comparison of stem and needle TCAA concentrations [ng (g dwt)$^{-1}$]

<table>
<thead>
<tr>
<th></th>
<th>T1 (Spray)</th>
<th>T2 (Soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stem</td>
<td>Needle</td>
</tr>
<tr>
<td>L0</td>
<td>48$^1$</td>
<td>144</td>
</tr>
<tr>
<td>L1</td>
<td>61</td>
<td>186</td>
</tr>
<tr>
<td>L2</td>
<td>277</td>
<td>315</td>
</tr>
</tbody>
</table>

(1 based on repeated single determination – see text)

The TCAA concentrations determined in the stems of T2 trees are much smaller than the needle concentrations, which suggests that the needles act as a sink for the TCAA applied to the soil. If TCAA is present in the xylem it is likely to be constantly diluted by the uptake of irrigation water. Conversely the stems of the T1 shoots have been directly exposed to TCAA and therefore contain much larger concentrations than the T2 stems. In L2T1 stems the concentrations are similar in magnitude to the needle

Figure 7.3; TCAA concentrations in stems of Sitka Spruce (October 1999)

(Each bar represents the mean ± standard deviation of 4 replicate analyses from a pooled sample of each exposure)
concentrations. A possible explanation for such high stem concentrations is that TCAA enters the stems, but does not directly pass into the xylem and so only slowly moves in the direction of the needles. Whether the T1 stem concentrations are eventually reduced by translocation or whether TCAA is permanently trapped in the stems is not known, nor is it known if the TCAA in the T1 stems is mobile. The location of TCAA within the stems i.e. bark or wood is not distinguished because the stems were fully homogenised prior to analysis, but it is clear that the burden of TCAA in these T1 trees is higher than T2 and so it possibly demonstrates a more efficient uptake pathway of TCAA by foliage spraying than via soil application. A final possibility is that TCAA is taken up via the stems and retained in situ. Subsequently degradation may not occur as the stems are not a metabolically active area of the tree. All of the above suggestions point to the existence of an uptake process through the stems. Recent work by Matucha et al. (2000a) has shown that if trees are treated via soil then 40% of the total TCAA found in trees is present in needles with only 10% in branches. The same 4-fold difference is observed in the T2 trees in this experiment (Table 7.3), particularly in L2T2.

An uptake pathway for TCAA through the stem surface has never previously been suggested in experiments using TCAA. Previous work which observed the uptake of TCAA through foliage suggested that it had occurred through the needle surface or by adsorption on it (Sutinen et al. 1995 & 1997), but this is thought unlikely in Sitka spruce (Section 7.4.6) and led to the investigation of other pathways. A route of uptake through the stem surface or bark is well known for other anionic species such as nitrate, which have been studied using $^{15}$N-labelled compounds (Eilers et al. 1992). Excess accumulation of $^{15}$N in stems compared to needles and roots was also found by Bowden et al. (1989). It was suggested that there was long term retention of the $^{15}$N in stems since it could not be removed by continued washings. Macklon et al. (1996) suggested that a direct uptake pathway had been previously reported by Tukey et al. (1962) and that the uptake of nitrogen species through stems was influenced by mass flow through splits, diffusion and exchange processes. Katz (1991) reported that uptake by bark was 5-times greater than that by needles. This evidence from the
literature supports the hypothesis that TCAA, which is also a simple anionic species, might also be taken into Sitka spruce via stem or bark and that this might even be a dominant uptake pathway in a forest where input of TCAA by cloud was ubiquitous.

The discovery of this phenomenon in greenhouse experiments means it is not clear whether these results can be extrapolated directly to a real forest environment. The seedlings used were smaller and younger than those typical of a mature forest and so the stems are more exposed and are less lignified, although older bark with more splits might favour uptake from cloudwater and stemflow. The openness of the canopy is untypical of the tightly enclosed branches in the canopy of the forested sites at Glentress Forest. The lack of true bark in these young plants might also make the uptake of TCAA more favourable than in a forest environment. For these reasons sampling and analysis of stems taken during routine sampling at Glentress Forest was carried out to determine the absolute TCAA concentrations and any patterns present with respect to the relative differences between stem and needles. Figure 7.4 shows the TCAA concentrations in stem material sampled at Dunsclair Heights and Venlaw on 27/10/99 in 6 and 4 year classes respectively.

The first observation about the results (Figure 7.4) is that there appear to be distinctly different patterns at the two sites. The stems at Dunsclair Heights show a marked decrease in TCAA concentration with year class whereas at Venlaw the concentrations remain fairly constant. By comparison at same sampling date (Figure 4.5 & 4.6) the pattern of needle concentrations at Dunsclair Heights is exactly the opposite of the pattern of concentrations in the stems. The absolute stem TCAA concentrations are low and relatively lower than the needles concentrations. Unlike the seedling experiments there does not appear to be a large accumulation of TCAA in the stems. This may be discussed in terms of the mode of wet input at each site.
Figure 7.4; TCAA concentrations in stem material at Glentress Forest
(Each bar is a mean ± standard deviation of triplicate analyses of a pooled sample)

At Venlaw there is little or no wet input from cloud so it is expected that input of TCAA through stems is small. The trees at Venlaw are well spaced and being younger their stems are relatively exposed, but without the cloud input this may have no effect. The pattern shows no increase in stem TCAA with increasing year class, which closely resembles the pattern for the needle concentrations, suggesting that the stem concentrations reflect TCAA uptake via the root pathway. At Dunslair Heights the pattern is the reverse of that for needles. The year C needle and stem TCAA concentrations are very similar, but in older year classes the concentrations begin to diverge with increases in needles and decreases in stems. The type of wet input experienced by the trees may also explain this.

At Dunslair Heights cloud is very common and may be present for long periods of time, which may lead to the uptake of TCAA through the stems as suggested by the similar year C concentrations. However, the canopy of the trees at Dunslair Heights is very dense and only the youngest branches protrude outwards. The ability of the cloud to come into contact with older branches is lessened, which would explain that as the older stems are progressively towards the interior of the tree the concentration determined in the stems decreases with year class (Figure 7.4). This suggests, as
discussed above, that the growing experiments are a good guide for possible uptake pathways but are not representative of real forests. The final implication of these results is that as the concentration of TCAA in stem material from Glentress Forest on 27/10/99 is very low, it does not reflect a recent uptake of a high concentration TCAA source i.e. cloud. The TCAA concentrations present in the stems may act as a better historical record of TCAA input to the forest than the needle concentrations. To prove this an assessment of the lifetime of TCAA in stems must be made.

7.4.3 Full analysis of needles (May 2000)

The trees were left untreated with TCAA solutions from October 1999 to May 2000 and overwintered in the glasshouse. The trees were kept healthy by irrigation with tap water, which was only required on average twice a month due to significantly reduced transpiration rates. The needles of the 1999 year class were sampled after bud burst at the beginning of the new growing season. The aim was to examine if the pattern of needle TCAA concentrations observed in Autumn had changed over the winter, a period when metabolism was greatly reduced. The measured needle TCAA concentrations are shown in Figure 7.5.

The needle TCAA concentrations have not been totally degraded and some features of the previous pattern still persist. The needle concentrations at L0 and L1 level are very similar to October 1999, and the needle concentrations present due to treatment with 10 ppb TCAA (L1) is indistinguishable from the controls (L0). The large concentrations in L0T2 B2, also evident in October 1999, are still present and remain unexplained. All the trees were watered evenly throughout the winter and it is not clear why this batch should be higher than the others. If the anomalous results from Batch 2 are omitted then the mean needle concentration in the controls in May 2000 [104 ± 22 ng (g dwt)^{-1} TCAA] are statistically the same to those in the controls in October 1999 [121 ± 16 ng (g dwt)^{-1} TCAA]. This is a surprising result as the input of TCAA from irrigation has been continued and so it appears that neither a significant increase, due to watering, nor a decrease, due to metabolism, has been observed. This may be explained by a steady state between the uptake of TCAA and
the metabolism in the needles, although in reality the actual uptake of TCAA has probably been very small.

**Figure 7.5; TCAA concentrations in C+1 needles of Sitka spruce (May 2000)**

*Each bar represents mean ± standard deviation on triplicate analyses of a pooled sample for each batch*

ANOVA of the TCAA concentrations measured in the needles in May 2000 (data not shown) indicates that no significant difference in TCAA concentrations between the mode of treatment or the level used, a change from the significant effect of Levels observed in October 1999. This suggests that TCAA has been removed possibly by metabolism in the needles. The change can be examined statistically by an ANOVA of the difference in needle concentrations between October and May (Table 7.4).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels</td>
<td>37865</td>
<td>2</td>
<td>18933</td>
<td>6.11</td>
<td>0.009</td>
<td>3.55</td>
</tr>
<tr>
<td>Treatments</td>
<td>5644</td>
<td>1</td>
<td>5644</td>
<td>1.82</td>
<td>0.194</td>
<td>4.41</td>
</tr>
<tr>
<td>Interaction</td>
<td>8224</td>
<td>2</td>
<td>4112</td>
<td>1.33</td>
<td>0.290</td>
<td>3.55</td>
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<tr>
<td>Within</td>
<td>55785</td>
<td>18</td>
<td>3099</td>
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<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>107518</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 7.4; Two-way ANOVA for change in needle TCAA concentrations between October 1999 and May 2000 in Sitka spruce seedlings**
Despite the large between-batch variability there is a statistical difference (P<0.01) in the needle TCAA concentrations for the level of the exposure. The concentrations in L2 trees fell to between 175 and 275 ng (g dwt)^{-1} TCAA in May, whereas the concentrations in October were between 250 and 350 ng (g dwt)^{-1} TCAA, showing that TCAA has been removed.

The conclusions from the analysis of needles in the growing experiments in May 2000 is that no statistical difference is observed due to treatment during the previous growing season because most of the fine structure present in the pattern from October has disappeared. This may be due to continued input of TCAA due to irrigation or metabolism within the needles, which has destroyed the marked differences previously observed. Another reason for the reduction of needle TCAA concentrations could be the translocation of TCAA to other parts of the trees. Matucha et al. (2000b) have suggested that TCAA can be translocated to either needles of older year class or to the roots. This may explain why even with constant input of TCAA the concentrations go down in the majority of seedlings. It would also help to explain why the removal of TCAA is different at the different levels, as the L0 seedlings have not shown any real decrease in needle concentrations, but L2 trees have decreased by up to 75 ng (g dwt)^{-1} TCAA. It is possible that the presence of higher needle TCAA concentrations promotes either metabolism or translocation. It would have been interesting to have examined freshly flushed year C (2000) needles for significant differences in TCAA, but unfortunately there was not enough material to do this and still leave the trees viable for continued experiments. The number of new needles present in the bud is determined during the previous year’s growing season so it would be interesting to see if the application of TCAA during formation in 1999 would affect concentrations in needles appearing in 2000.

7.4.4 Soil TCAA analysis (October 1999)

The concentrations of TCAA in the soil in the pots were determined 26 days after the final treatment of the trees. The TCAA concentrations (shown in Figure 7.6) give indications as to the speed and efficiency of TCAA uptake by the seedlings and its
lifetime due to degradation in the soil. The soil concentrations relative to dry weight are likely to be directly proportional to results expressed relative to soil total organic matter (TOM) given the initial homogenisation of soils at the start of the experiment. The concentrations reported were determined in a homogenised, pooled sample of soil cores taken from each pot of the batch.

Figure 7.6; Soil TCAA concentrations in growing experiment (October 1999)
(Each bar represents mean ± standard deviation on triplicate analyses of a pooled sample for each batch)

The most striking pattern observed from the data in Figure 7.6 is that there are higher concentrations in T1 soils than T2 soils at both L1 and L2. The pattern is reversed for the control soils as shown by the highly significant (P<0.02) level x treatment interaction term (Table 7.5). This is counter-intuitive as it was expected that T2 soils would contain some residual TCAA from soil treatments. Instead, the soil that never had TCAA directly applied to it displays statistically significant (P<0.05) higher concentrations (see Table 7.5). There is also no effect of Level on the soil TCAA concentration as observed for the needles. This suggests that the soil TCAA concentrations are maintained at approximately a constant level by some process. The TCAA determined in the soil of the outside trees shows similar concentrations to the L1T2 and L2T2 trees. This suggests no enhancement of the TCAA concentration has
occurred in the soil after 5 months of treatment with TCAA standards and irrigation with tap water. This would lead to the conclusion that either:

- The applied TCAA has been taken up by the trees with subsequent storage and/or metabolism in plant parts, or
- Any residual TCAA has been broken down in the soil.

The close agreement between batches of the same treatment suggests that the ongoing uptake processes in the trees are uniform between the batches and are reproducible. From comparison of Figures 7.2 & 7.6 it is not possible to correlate the size of the TCAA concentrations in needles to the levels determined in the analogous soils. Another suggestion for the higher concentrations determined in T1 soils could be the translocation of TCAA within trees and the subsequent exudation by the roots. The differences between the soils are quite small and may be due to slight differences in uptake of irrigation water, which was observed during winter.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels</td>
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<td>2</td>
<td>11.48</td>
<td>0.69</td>
<td>0.515</td>
<td>3.55</td>
</tr>
<tr>
<td>Treatments</td>
<td>74.69</td>
<td>1</td>
<td>74.69</td>
<td>4.48</td>
<td>0.049</td>
<td>4.41</td>
</tr>
<tr>
<td>Interaction</td>
<td>174.71</td>
<td>2</td>
<td>87.35</td>
<td>5.24</td>
<td>0.016</td>
<td>3.55</td>
</tr>
<tr>
<td>Error</td>
<td>300.34</td>
<td>18</td>
<td>16.69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>572.69</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 7.5: Two-way ANOVA for soil TCAA concentrations in Sitka spruce seedlings (October 1999)

7.4.5 Direct effects of TCAA treatment on Sitka spruce

7.4.5.1 Growth measurements

The measurement of both height and root collar of all the trees were performed at the start and the end of the experiment to establish if any effect on growth could be observed after treatment at environmental TCAA concentrations. Due to the large variation in natural parameters such as tree height, no statistical differences were observable between the treatments for either of the two direct measurements made (data not shown). For a more sensitive measure the measurements of root collar and
height were combined and converted to a volume by assuming the tree stem to be a cone. Using Equation 7.1 the stem volume was obtained from the measurements.

\[ v = \frac{1}{3} \pi r^2 h \]  

Equation 7.1

where;
\( v = \text{volume of stem (cm}^3\)\)
\( r = \text{root collar \[radius (cm)\]}\)
\( h = \text{tree height (cm)}\)

The percentage change in volume was used as a probe of any physiological effect. The full results are shown in Table 7.6 with the results from a two-way ANOVA of differences between treatments in Table 7.7.

<table>
<thead>
<tr>
<th></th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Batch 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT1</td>
<td>110±35</td>
<td>180±65</td>
<td>85±18</td>
<td>96±40</td>
</tr>
<tr>
<td>LOT2</td>
<td>120±45</td>
<td>130±89</td>
<td>140±88</td>
<td>76±41</td>
</tr>
<tr>
<td>L1T1</td>
<td>140±47</td>
<td>77±50</td>
<td>130±100</td>
<td>200±130</td>
</tr>
<tr>
<td>L1T2</td>
<td>130±53</td>
<td>160±70</td>
<td>160±110</td>
<td>140±77</td>
</tr>
<tr>
<td>L2T1</td>
<td>79±43</td>
<td>110±73</td>
<td>55±27</td>
<td>130±64</td>
</tr>
<tr>
<td>L2T2</td>
<td>170±55</td>
<td>130±62</td>
<td>76±64</td>
<td>65±18</td>
</tr>
</tbody>
</table>

Table 7.6; Mean percentage change in stem volume during seedling experiment

(\text{Error ranges are standard deviations of 5 seedling measurements per batch})

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>(F)</th>
<th>P-value</th>
<th>(F) crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level</td>
<td>6582.33</td>
<td>2</td>
<td>3291.17</td>
<td>2.24</td>
<td>0.136</td>
<td>3.55</td>
</tr>
<tr>
<td>Treatment</td>
<td>425.04</td>
<td>1</td>
<td>425.04</td>
<td>0.29</td>
<td>0.598</td>
<td>4.41</td>
</tr>
<tr>
<td>Interaction</td>
<td>377.33</td>
<td>2</td>
<td>188.67</td>
<td>0.13</td>
<td>0.880</td>
<td>3.55</td>
</tr>
<tr>
<td>Within</td>
<td>26485.25</td>
<td>18</td>
<td>1471.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33869.96</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.7; Two-way ANOVA of change in seedling stem volume results

The variation in the change in volume is high within batches (high standard deviations) and between batches of the same treatment and so only the most obvious
differences would be statistically significant. It seems that the change in stem volume is not a sensitive indicator of TCAA application at these environmental concentrations. It is also possible that at these low doses the Sitka spruce seedlings experience no physiological effects. However, the failure to observe any inhibition of growth after treatment with TCAA has implications for measurements at Dunslair Heights. The measurement of needle concentrations in the seedlings in excess of 300 ng (g dwt)$^{-1}$ TCAA is far higher than reported in the literature or measured at Dunslair Heights in any year class of needles (Chapter 4). If even at these higher needle concentrations no negative effects on tree growth are experienced then it is questionable if TCAA will ever have any implications for tree health at the measured environmental concentrations. It is possible that the effects of these concentrations may not be realised until the next growing season, which is why these experiments should be continued.

7.4.5.2 Tree mortality

Matucha (Pers. Comm.) has stated that in an experiment with $^{14}$C-labelled TCAA on nursery seedlings initially no physiological effects of TCAA was observed. An interesting finding was that during the growing season after the treatment period the seedlings exhibited signs of forest decline, with bad necrosis and loss of needles and eventual death. However, this was only performed with one exposed and one control seedling and so is not statistically significant. To investigate this phenomenon our trees were visually examined for similar forest decline indicators in August 2000.

In August 2000, of the original 120 seedlings, only 2 had died. During the winter period the needles appeared bleached which was attributed to the trees becoming pot bound. The trees were re-potted and subsequently the new year C needles (2000) have appeared very healthy and green. This measure of tree health is clearly very subjective, but it has been concluded that TCAA has not had an effect on tree mortality during the treatment period or in the subsequent year.
7.4.6 Partitioning experiments

It has been questioned above whether aqueous TCAA can easily pass into the matrix of needles through the stomata due to its form as a simple anion. These partitioning experiments were designed to test whether the immersion of needles in low and high concentration solutions of TCAA would lead to uptake by the needles. Two experiments were performed, one on a long time-scale (1 day) and the other on a shorter time-scale (2 hours). These partitioning experiment were designed to mimic the conditions of the tree growing experiments. The results of the first partitioning experiment (over 1 day) performed at neutral pH are shown in Figure 7.7.

![Figure 7.7: The effect of length of exposure to TCAA solutions on needle concentrations in laboratory partitioning experiments](image)

*Figure 7.7: The effect of length of exposure to TCAA solutions on needle concentrations in laboratory partitioning experiments*  
*Error bars represent standard deviations on triplicate analysis. The time zero results are direct measurements of needle TCAA concentration at the start of the experiment*

When needles containing low concentrations were used the analytical uncertainties were large. Some of the results presented represent 1 or 2 replicates. No firm conclusions can be drawn from this experiment due to the poor reproducibility, but
some general points can be stated. The initial TCAA concentrations in the needles [7 ng (g fwt)⁻¹ TCAA] were sufficiently low that any uptake of TCAA should be easily distinguished. After exposure similar concentrations are found in the 10 ppb (L1) and 100 ppb (L2) treated needles showing no fast uptake process of TCAA. After 1 hour there appears to have been an increase in both L0 and L2, which may represent the end of resistance to uptake by stomata. However, subsequent exposure to TCAA solutions for as long as 24 hours does not result in an uptake of TCAA which is significantly different to the controls. This would also suggest that longer term treatment with TCAA does not result in a direct uptake through the needle surfaces. The implication for the growing experiments is that a single treatment by spraying the needles is unlikely to lead to significant foliar uptake unless the process of evaporation leads to very much higher concentrations and subsequent uptake.

The second experiment used exposure for up to 2 hours with solutions at pH 4. At this pH more TCAA is present in its non-dissociated form, which may influence uptake. The exposure of needles to TCAA was performed over 5 time periods using 2 concentrations (10 and 100 ppb) and a control of deionised water. The results are shown in Figure 7.8.

The results seem to show a gradual increase in partitioning of TCAA into needles up to 30 minutes exposure and then a subsequent decrease in concentration with time. It should be said that no significant differences are seen between the L1 and L2 trees and the controls (except 90 min.). This would suggest that no partitioning of TCAA into the needles has occurred even at high TCAA concentrations (100 ppb). An unusually high result has been measured for the control after 90 minutes exposure which has been explained by some sort of contamination as similar increases are not seen in the needles exposed to 10 and 100 ppb standard solutions.
Figure 7.8: The effect of exposure time to TCAA solutions on needle concentrations in laboratory partitioning experiments.

(Error bars represent standard deviations on triplicate analysis. The time zero results are direct measurements of needle TCAA concentration at start of experiment)

The conclusions that can be drawn from these experiments is that TCAA is not easily taken up by needles after direct contact of aqueous solution with their surfaces. This would imply that the growing experiments would be unlikely to show uptake after exposure to foliage only. Uptake studies on needles using nitrate and ammonium have found that the processes responsible are dictated by the charge on the ion, the charge on the cuticular material (predominantly negative) and the internal concentrations of the ions (Macklon et al. 1996). For nitrate the cuticle presents less of a barrier to inward diffusion than for cations since negatively charged ions will not be retained by negative exchange sites. The rate limiting step is most likely to be entry across the plasmalemma of leaf cells against the electrochemical diffusion gradient. There seems no logical reason why a similar process should not occur for TCAA, but it has not be observed in these experiments. One reason why uptake by needles may not be seen is that uptake could be an active process and when the needles are removed from living trees this may cease. Inward partitioning has not
occurred, even to a small extent, after exposure to high concentrations for up to 24 hours so it seems unlikely to occur unless by an active uptake process.

The seedling experiments have shown that there is a significant role of bark/stem in the uptake and translocation of TCAA to needles. After TCAA solution is applied to the needles of the seedlings the droplets evaporate with the possibility of an accumulation of a large concentrations of TCAA on the needles. The large concentration gradient between the needle surface and the interior may be the driving force for transport across the leaf cuticle, which is not simulated in the laboratory partitioning experiment. A direct comparison between these laboratory partitioning experiments and the greenhouse experiments may not be possible as the needles on the greenhouse grown seedlings may have very different cuticle development from needles on mature trees from outdoors (Cape & Percy 1993).

### 7.4.7 Isolation and measurement of Glutathione-S-transferase (GST)

#### 7.4.7.1 Results

The results from the measurements of the protein content (mg ml\(^{-1}\)) of the fine roots and current year needles of the seedlings performed by GSF (Germany) are shown in Table 7.8 & 7.9. Measurements of GST activity using DCNB as the xenobiotic substrate (nmol min\(^{-1}\) ml\(^{-1}\)) are shown in Table 7.10. The measurements of GST activity using CDNB and NBOC, and peroxidase (POX) activity using guajacol in the needles were incomplete and so are not presented here.

<table>
<thead>
<tr>
<th></th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Batch 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>L0T1</td>
<td>0.194 ± 0.001</td>
<td>0.300 ± 0.004</td>
<td>0.320 ± 0.009</td>
<td>0.168 ± 0.009</td>
</tr>
<tr>
<td>L0T2</td>
<td>0.464 ± 0.041</td>
<td>0.379 ± 0.027</td>
<td>0.436 ± 0.005</td>
<td>0.363 ± 0.013</td>
</tr>
<tr>
<td>L1T1</td>
<td>0.325 ± 0.142</td>
<td>0.165 ± 0.016</td>
<td>0.136 ± 0.003</td>
<td>0.154 ± 0.082</td>
</tr>
<tr>
<td>L1T2</td>
<td>0.195 ± 0.003</td>
<td>0.275 ± 0.016</td>
<td>0.375 ± 0.012</td>
<td>0.091 ± 0.001</td>
</tr>
<tr>
<td>L2T1</td>
<td>0.232 ± 0.015</td>
<td>0.183 ± 0.008</td>
<td>0.186 ± 0.004</td>
<td>0.147 ± 0.017</td>
</tr>
<tr>
<td>L2T2</td>
<td>0.256 ± 0.015</td>
<td>0.145 ± 0.001</td>
<td>0.210 ± 0.004</td>
<td>0.175 ± 0.001</td>
</tr>
</tbody>
</table>

Table 7.8; Protein content measurements in seedling needles (mg ml\(^{-1}\))

(Operator ranges are standard deviations of duplicate measurements of crude extract)
Table 7.9; Protein content measurements in seedling fine roots (mg ml\(^{-1}\))

<table>
<thead>
<tr>
<th></th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Batch 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT1</td>
<td>0.200 ± 0.001</td>
<td>0.243 ± 0.009</td>
<td>0.305 ± 0.008</td>
<td>0.146 ± 0.001</td>
</tr>
<tr>
<td>L0T2</td>
<td>0.418 ± 0.018</td>
<td>0.363 ± 0.005</td>
<td>0.137 ± 0.023</td>
<td>0.243 ± 0.009</td>
</tr>
<tr>
<td>L1T1</td>
<td>0.354 ± 0.054</td>
<td>0.158 ± 0.004</td>
<td>0.079 ± 0.006</td>
<td>0.078 ± 0.004</td>
</tr>
<tr>
<td>L1T2</td>
<td>0.110 ± 0.008</td>
<td>0.176 ± 0.021</td>
<td>0.263 ± 0.021</td>
<td>0.045 ± 0.010</td>
</tr>
<tr>
<td>L2T1</td>
<td>0.136 ± 0.003</td>
<td>0.125 ± 0.006</td>
<td>0.131 ± 0.010</td>
<td>0.105 ± 0.002</td>
</tr>
<tr>
<td>L2T2</td>
<td>0.209 ± 0.008</td>
<td>0.125 ± 0.009</td>
<td>0.157 ± 0.004</td>
<td>0.197 ± 0.071</td>
</tr>
</tbody>
</table>

Table 7.9; Protein content measurements in seedling fine roots (mg ml\(^{-1}\))

(Error ranges are standard deviations of duplicate measurements of crude extract)

Table 7.10; GST activity measurements in needles with DCNB as xenobiotic substrate (x10\(^{-3}\) nmol min\(^{-1}\) ml\(^{-1}\))

<table>
<thead>
<tr>
<th></th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Batch 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT1</td>
<td>1.10 ± 0.17</td>
<td>0.63 ± 0.15</td>
<td>1.33 ± 0.06</td>
<td>1.83 ± 0.25</td>
</tr>
<tr>
<td>L0T2</td>
<td>0.67 ± 0.12</td>
<td>1.13 ± 0.06</td>
<td>0.57 ± 0.23</td>
<td>0.50 ± 0.00</td>
</tr>
<tr>
<td>L1T1</td>
<td>0.80 ± 0.10</td>
<td>0.57 ± 0.15</td>
<td>1.00 ± 0.20</td>
<td>1.33 ± 0.21</td>
</tr>
<tr>
<td>L1T2</td>
<td>0.97 ± 0.29</td>
<td>0.60 ± 0.10</td>
<td>0.43 ± 0.40</td>
<td>0.67 ± 0.21</td>
</tr>
<tr>
<td>L2T1</td>
<td>3.77 ± 0.06</td>
<td>0.30 ± 0.00</td>
<td>0.27 ± 0.15</td>
<td>0.53 ± 0.12</td>
</tr>
<tr>
<td>L2T2</td>
<td>0.83 ± 0.06</td>
<td>0.43 ± 0.06</td>
<td>0.13 ± 0.06</td>
<td>0.27 ± 0.06</td>
</tr>
</tbody>
</table>

Table 7.10; GST activity measurements in needles with DCNB as xenobiotic substrate (x10\(^{-3}\) nmol min\(^{-1}\) ml\(^{-1}\))

(Error ranges are standard deviations of triplicate measurements of crude extract)

7.4.7.2 Discussion of GST measurements

The measurements of protein in both roots and needles were analysed statistically using ANOVA and Tukey's Studentised Range Test. The ANOVA is split into its contributions of degrees of freedom and the SAS statistical model is run. The full ANOVA with all the interactions was calculated, and interaction terms removed sequentially in order of their contribution to the sum of squares. If there is no significance to the interaction terms they are removed and the model finally run with only the combination of main effects which are significant in the initial ANOVA. The
remaining degrees of freedom are used as error terms in evaluating significant difference (see Appendix A for a worked example of ANOVA and Tukey’s Test). The main significant results of the ANOVA are presented for needle protein content (Table 7.11, 7.12 & 7.13), root protein content (Table 7.14, 7.15 & 7.16) and GST activity with DCNB (Table 7.17, 7.18 & 7.19).

**Protein content in the needles of seedlings**

Table 7.11 shows that there is a significant effect of Batch and Level of treatment on the protein content measured in the Year C needles (P<0.02). Batch number is directly related to the size of the seedlings with Batch 1 being the tallest and Batch 4 being the smallest seedlings at the start of the experiment. Table 7.12 shows that the protein content is directly related to the seedling size, with Batch 1 significantly different to Batch 4. Conversely, Table 7.13 shows an inverse relationship between the protein content and the level of TCAA treatment of the seedlings with the control seedlings (L0) containing significantly higher protein contents than Level 2 seedlings (100 ppb). This suggests that larger trees have higher protein contents than smaller ones, which may enable them to detoxify xenobiotics more effectively by conjugation. The increased protein content in larger trees is not related to higher concentrations of TCAA as no relationship between Batch and TCAA concentrations has been found (Table 7.2a & 7.2b). The relationship between Level and Protein content can be explained in terms of the properties of TCAA. The significant reduction in protein in seedlings after application with 100 ppb TCAA suggests that its herbicidal properties have had an effect in reducing protein content even after a relatively short term application at close to environmental concentrations. This finding has been reported previously (Plümacher & Schröder 1994), where the TCAA concentrations in needles were inversely correlated to the protein content (P<0.025). This was explained by the precipitating effects of TCAA. This would result in lower protein content in trees, which would reduce their ability to conjugate and thus detoxify xenobiotics. The mean protein contents are slightly higher in soil treated (T2) seedlings, but this is not significant.
Table 7.11; Two-way ANOVA results for protein content in needles

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>ANOVA SS</th>
<th>Mean Square</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>3</td>
<td>0.07907081</td>
<td>0.02635694</td>
<td>3.93</td>
<td>0.0147</td>
</tr>
<tr>
<td>Level</td>
<td>2</td>
<td>0.06449293</td>
<td>0.03224647</td>
<td>4.81</td>
<td>0.0132</td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td>0.28159637</td>
<td>0.00670468</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.12; Tukey grouping by Batch for needle protein content

(Means with the same letter are not significantly different)

<table>
<thead>
<tr>
<th>Tukey grouping</th>
<th>Mean Protein (mg ml⁻¹)</th>
<th>n</th>
<th>Batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.27759</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>A/ B</td>
<td>0.24114</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>A/ B</td>
<td>0.22898</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>0.16510</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 7.13; Tukey grouping by Level for needle protein content

(Means with the same letter are not significantly different)

<table>
<thead>
<tr>
<th>Tukey grouping</th>
<th>Mean Protein (mg ml⁻¹)</th>
<th>n</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.27833</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>A/ B</td>
<td>0.21459</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>0.19169</td>
<td>16</td>
<td>2</td>
</tr>
</tbody>
</table>

Protein content in the fine roots of seedlings

Table 7.14 shows similar patterns in root protein content as observed for needle protein content. Increased root protein content with the size of the seedlings is demonstrated in Table 7.15, with significantly larger protein content in Batch 1 than in Batch 4. This is expected to be related to the herbicidal properties of TCAA as mentioned in the previous section. Table 7.16 shows that there is also a significant difference between the exposed seedlings (L1 & L2) and the control seedlings (L0), with lower mean protein content after TCAA treatment at either level. This suggests that both the application of TCAA to the soil or the foliage at concentrations greater than 10 ppb results in a significant reduction in protein content in the roots. This
finding supports the hypothesis that exposure of trees to TCAA at close to environmental concentrations results in damage to the detoxification ability of trees. A discussion of the changes in GST activity in the seedlings is in the next section.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>ANOVA SS</th>
<th>Mean Square</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>3</td>
<td>0.06492729</td>
<td>0.02164243</td>
<td>3.60</td>
<td>0.0212</td>
</tr>
<tr>
<td>Level</td>
<td>2</td>
<td>0.11568786</td>
<td>0.05784393</td>
<td>9.63</td>
<td>0.0004</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.01224963</td>
<td>0.01224963</td>
<td>2.04</td>
<td>0.1608</td>
</tr>
<tr>
<td>Error</td>
<td>41</td>
<td>0.24626453</td>
<td>0.00600645</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.14; Two-way ANOVA results for protein content in roots

<table>
<thead>
<tr>
<th>Tukey grouping</th>
<th>Mean Protein (mg ml⁻¹)</th>
<th>n</th>
<th>Batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.23763</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>A/ B</td>
<td>0.19838</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>A/ B</td>
<td>0.17874</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>0.13551</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 7.15; Tukey grouping by Batch for root protein content

(Means with the same letter are not significantly different)

<table>
<thead>
<tr>
<th>Tukey grouping</th>
<th>Mean Protein (mg ml⁻¹)</th>
<th>n</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.25676</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0.15784</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>0.14809</td>
<td>16</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 7.16; Tukey grouping by Level for root protein content

(Means with the same letter are not significantly different)

**GST activity in the needles of seedlings**

GST activity has been measured in the seedlings using DCNB as a xenobiotic substrate. Table 7.17 shows that significant changes are related to the Batch (size) and the Treatment type of the seedlings. Table 7.18 suggests that Batch 1 is
significantly different to Batch 2 and 3, but not to Batch 4. The reason for this
difference is not understood as no similarly significant differences exist for the TCAA
ccentration or protein content of needles. The pattern in the batches may be
erroneous and related to the low response of the assay with DCNB or the relatively
high standard deviations of some of the triplicate measurements. It has been shown
previously (Schröder et al. 1997) that the use of CDNB as a xenobiotic substrate
produced higher GST activities than using DCNB. It has been suggested that this is
because conjugation of DCNB is related to different GST isoenzymes.

Table 7.19 shows a highly significant (P=0.001) difference in GST activity between
the treatment type of the seedlings. The mean GST activity is significantly higher
after treatment via the foliage. No interaction terms or any significance of level
means that the effect of spraying the needles on GST activity is even observed in the
control seedlings. This suggests that the action of wetting the needle surfaces either
with water or TCAA solutions enhances GST activity and not the application of
TCAA. However, it should be remembered that the TCAA concentration of control
deionised water (5 ppb) is significant relative to environmental concentrations and that
GST activity may be enhanced by the addition of this low TCAA concentration. This
is in agreement with the suggestion that uptake of TCAA is more efficient by foliage
spraying than by direct application to the soil (Section 7.4.2). The effect of the small
concentration of TCAA in deionised water might only alter enzyme activity if
efficiently absorbed and transported to the needles. It has been reported by (Schröder
et al. 1997) that GST activity was induced in all TCAA samples of Pinus sylvestris L.
seedlings and so supports the findings of this experiment.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>ANOVA SS</th>
<th>Mean Square</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>3</td>
<td>6.55 x 10^-6</td>
<td>2.18 x 10^-6</td>
<td>5.26</td>
<td>0.0026</td>
</tr>
<tr>
<td>Level</td>
<td>2</td>
<td>4.38 x 10^-7</td>
<td>2.19 x 10^-7</td>
<td>0.53</td>
<td>0.5922</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>4.91 x 10^-6</td>
<td>4.91 x 10^-6</td>
<td>11.82</td>
<td>0.0010</td>
</tr>
<tr>
<td>Error</td>
<td>65</td>
<td>2.70 x 10^-5</td>
<td>4.2 x 10^-7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.17; Two-way ANOVA results for GST activity (DCNB) in needles
### Table 7.18: Tukey grouping by Batch for GST activity (DCNB) in needles
(Means with the same letter are not significantly different)

<table>
<thead>
<tr>
<th>Tukey grouping</th>
<th>Mean GST activity (nmol min⁻¹ ml⁻¹)</th>
<th>n</th>
<th>Batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.36 x 10⁻³</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>A/ B</td>
<td>8.56 x 10⁻⁴</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>6.22 x 10⁻⁴</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>6.11 x 10⁻⁴</td>
<td>18</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 7.19: Tukey grouping by Treatment for GST activity (DCNB) in needles
(Means with the same letter are not significantly different)

<table>
<thead>
<tr>
<th>Tukey grouping</th>
<th>Mean GST activity (nmol min⁻¹ ml⁻¹)</th>
<th>n</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.12 x 10⁻³</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>6.00 x 10⁻⁴</td>
<td>36</td>
<td>2</td>
</tr>
</tbody>
</table>

### 7.5 Conclusions

Uptake of TCAA has been shown to occur via two pathways, one via the root network and the other above ground. The root pathway has been well characterised by uptake via the transpiration stream with storage in the needles. The above ground pathway has previously been assumed to be adsorption of TCAA to needle surface, but the vigorous washing used prior to analysis suggests that the TCAA measured in the needles has been assimilated into the matrix of the needles. The analysis of the stem material has shown significantly higher concentrations in the stems of seedlings treated with a TCAA mist compared to those with soil applications, implying that uptake occurs through the bark of stems and branches. This pathway has previously been suggested for the uptake of nitrate by Red Spruce, after studies with ¹⁵N labels (Bowden et al. 1989). Rapid translocation of TCAA from stems to needles is possible. The uptake of TCAA through the above ground route has shown the same needle TCAA concentrations as soil applications, despite poor capture efficiency of the canopy. This pathway appears to be more efficient at uptake than by direct addition to the soil, leading to the suggestion that TCAA may be degraded or
adsorbed in the soil prior to root uptake. Partitioning experiments have indicated that the uptake of TCAA directly by needles is unlikely to occur unless active processes are involved.

Significant uptake of TCAA has been observed after treatment with 10 ppb and 100 ppb TCAA solutions. The concentrations exhibited by needles in this experiment [up to 370 ng (g dwt)\(^{-1}\) TCAA] are higher than any previously measured in the field and were significantly higher than the 35 and 60 ng (g fwt)\(^{-1}\) TCAA measurements presented by Sutinen \textit{et al.} (1997). The concentration of 10 ppb used for spraying is very close to the maximum concentrations in cloud water (Figure 6.3). Previous studies by Sutinen \textit{et al.} (1995 & 1997) have used far higher concentrations (500 and 1000 ppb) than experienced in nature and has led to damage to surface waxes. The actual concentrations measured in branches at Glentress Forest have been shown to be small, suggesting that uptake via the stems of mature trees is not as important in the field as suggested by the seedling experiments.

Low levels of residual TCAA have been detected in the soil after the exposure period with no significant differences between soil and foliage applied treatments nor between treatment levels. This demonstrates either very efficient uptake by seedlings or very rapid degradation. The analysis of needle concentrations during the subsequent growing season (May 2000) showed that high concentrations of TCAA [175 to 275 ng (g dwt)\(^{-1}\)] still remained in the needles of the L2 treated seedlings, but this was reduced relative to the previous measurements in October 1999. Degradation or translocation of TCAA elsewhere in the trees was assumed to be responsible.

No significant physiological changes have been observed from the application of TCAA even at the higher concentration. Measurements of root collar and height have shown no significant differences between treatments and the controls. Visible symptoms of forest decline or tree mortality have not been observed either during the exposure period or during the following season. However, the measurements of
enzymatic activity and protein content in the needles and roots of the seedlings have shown that seedling exposure to TCAA has resulted in biochemical changes related to detoxification processes. Some significant changes in protein content have been observed related to the seedling size and the TCAA level used, which is associated with the herbicidal properties of TCAA. Limited information is currently available about the GST activity in the seedlings, but using DCNB as a substrate the activity has been shown to be affected by the treatment type. The lack of effect of the application level on GST activity means that either the changes in GST activity are related to the process of wetting the needles or that GST activity is enhanced by the uptake of very low concentrations of TCAA, which were present in the deionised water used on the control seedlings.

The exposure experiments will be continued using the same group of trees to further investigate the effects of TCAA on Sitka spruce. A significant improvement in the experiment would be to use a source of irrigation with lower TCAA contamination i.e. stream or rain water. The use of tap water introduced a significant amount of TCAA to the experiment and meant that changes in needle TCAA concentrations due to the exposure at the L1 level was very difficult to distinguish from the controls. Continued measurements of the enzyme activity of the needles and roots sampled in October 1999 are to be made, particularly to investigate the plant response to oxidative measurements (Levine et al. 1994). Complete determination of peroxidase and GST activity will be used to investigate the biochemical effect of TCAA on seedlings.
Chapter 8

A mass balance of TCAA in a forest ecosystem at Glentress Forest

8.1 Introduction

Mass balance calculations are used to estimate the fluxes and reservoirs in a specific environmental compartment or system. In the literature two attempts have been made to perform mass balance calculations to estimate the cycling of TCAA within the environment and also to investigate the potential formation processes in the soil. The main aim of this mass balance calculations is to determine whether there is evidence for a natural formation of TCAA in the soil, as suggested by Hoekstra et al. (1999a), or whether the measured concentrations can be explained by the atmospheric deposition of TCAA, which is thought to originate from its chlorinated solvent precursors (Frank et al. 1991). Both sides of the debate are concisely summarised in a short exchange of contrary views on this research topic (Juuti & Hoekstra 1998; Frank et al. 1999).

If such fundamental conclusions are to be drawn from the mass balance calculations in the literature then clearly it is important to use the most accurate and representative TCAA measurements for the model. This is a very difficult task when considering data from different sources, which may comprise results from different sites in countries with various land use, climate, soil type and tree species using different analytical methodologies. Sometimes details of these variables are not fully included in the literature. It is also debatable whether measurements in the literature from different sources with differing conditions can be combined to elucidate trends or if mean concentrations should be used in mass balance estimates.

The only previously published attempt at a complete mass balance calculation was by Hoekstra et al. (1999a). This attempted to combine results from their own
research with those from other European studies to look for TCAA formation processes in the top soil layer and not a whole catchment. Tentative evidence was presented that TCAA was formed in the soil, but this conclusion needed the caveat of the large uncertainties in the measured input data. The reason for this was the decision to include all the available data, which constituted a relatively small set, rather than to restrict the data to a particular ecosystem. The fact that the data were collated from the literature rather than from a well understood data-set meant that attempts to reduce the uncertainties by rejecting apparent outliers was not possible. However, the overall approach taken by Hoekstra \textit{et al.} (1999a) was logical and well thought out and it is on this zero-dimensional box model approach that the Dunslair Heights mass balance is based, with the goal to replicate and improve the calculation with the use of more coherent input data from a single site.

There is a wealth of data available from the research conducted for this thesis at Dunslair Heights (Chapters 4, 5 & 6). All these data have been systematically collected including descriptions of sampling locations, meteorological conditions and sampling protocol. The application of these data to mass balance calculations is easier than using data from the literature, which are sometimes less complete and cannot be critically assessed. No other study in the literature has produced such a temporally extended study as this one of the environmental compartments of interest at the same sampling location. It is important to understand temporal variations in the concentrations of TCAA in any compartments caused by natural processes or atmospheric input. The availability of these data means a mass balance can be performed, assuming a generalised stand of trees at this specific site using measured TCAA concentrations and actual meteorological conditions. Some measurements have been made in this study which, particularly for soil, other analytical methods cannot produce. The differentiation between bound and non-bound TCAA in soil, which is not possible by an extraction and derivatisation methodology, has been described in Chapter 5. The concentrations of TCAA in air, particulate, cloudwater and stem material have also been measured extensively for the first time and hence were not available for the previous models. Some data were not available at Dunslair Heights, particularly throughfall and seepage \textit{via} groundwater, as these were not
covered in the scope of the study, but appropriate estimates will be made for the missing data. It is hoped these calculations will be relevant for other researchers in the TCAA field and that application of the results from this site to other European sites is possible. A final aim of the calculations was to identify whether the large sources of TCAA from soil production described by Hoekstra *et al.* (1999a) are realistic for the Dunslair Heights site.

8.2 Method

8.2.1 Mass balance model

In order to determine the mass balance of TCAA in the forest ecosystem at Dunslair Heights, a simple zero-dimension box model is used. The model is based on the time period April 1999 to April 2000 representing a full growing season in which measurements have been made in all the relevant media. The box model for TCAA in this catchment (Figure 8.1) is constructed in a similar way to that of Hoekstra *et al.* (1999a), where the burden of TCAA in each of the reservoirs or environmental compartments is calculated and the input and output fluxes of TCAA through the system is estimated where possible. The fact that all the data have been collected from the same site means that no assumptions need to be made as to what data should be included, with one notable exception. The measured concentrations of TCAA in the soil are exceptionally variable, both horizontally and vertically and also between tree stands and open moorland at Dunslair Heights. The simple use of an overall average value across all the samples is therefore not appropriate, and alternative approaches will be addressed about which concentrations are representative of a stand of trees. This will be addressed at the appropriate point in the discussion.

If TCAA is only present in the environment as a result of the atmospheric photodegradation oxidized as a result of the atmospheric photo-oxidation of its C₂-chlorinated precursors, such as 1,1,1-trichloroethane and tetrachloroethene, then the inputs to the box/ volume under consideration must equal the net rate of accumulation of TCAA in all reservoirs minus the outputs from the volume. If there is a discrepancy between the sums then there must be other sources or processes occurring e.g. natural production of TCAA in soil. There are certain
Figure 8.1; Schematic diagram of the box model
scenarios that the mass balance may produce, the implications of which are shown below;

*If input flux >> output flux* then there is a net sink of TCAA within the catchment. This might be due to accumulation in the soil or the trees or its degradation therein.

*If input flux << output flux* there is a net source of TCAA within the catchment, such as natural production processes in the soil.

*If time-integrated external fluxes >> total catchment burden* then the residence time in the catchment is probably short. This would mean that the processes in the catchment such as adsorption by the soil or degradation processes are unlikely to be significant and TCAA cycling is probably regulated by the external fluxes.

*If time-integrated external fluxes << total catchment burden* then TCAA cycling is likely to be dominated by internal catchment processes.

The results from the mass balance calculations are assessed with respect to the above criteria so that conclusions about the Dunslair Heights catchment can be made.

### 8.2.2 Model input parameters

#### 8.2.2.1 Atmospheric input

The input fluxes have been calculated from the wet and dry deposition data reported in Chapter 6. The input flux ($F_{\text{input}}$) can be represented as follows,

$$ F_{\text{input}} = F_{\text{wd}} + F_{\text{dd}} \quad \text{Equation 8.1} $$

where $F_{\text{wd}}$ is the wet deposition flux and $F_{\text{dd}}$ is the dry deposition flux, all expressed in $\mu g \ m^{-2} \ yr^{-1}$. The total wet deposition flux ($F_{\text{wd}}$) can similarly be represented by Equation 8.2,

$$ F_{\text{wd}} = F_{\text{cloud}} + F_{\text{rain}} \quad \text{Equation 8.2} $$

where $F_{\text{cloud}}$ is the input flux from cloudwater and $F_{\text{rain}}$ is the input flux from rainwater. These are taken directly from the wet deposition data in Chapter 6. Similarly the dry deposition flux can, in principle, be separated into the contributions from gas-phase and particulate-bound TCAA. However, the
monitoring at Dunslair Heights measured the total TCAA air concentrations so no information is available on the relative contributions from each. To accommodate this a deposition velocity to forest of 1 cm s\(^{-1}\) has been assumed to estimate F\(_{dd}\). All of the measurements of these input parameters (Table 6.14) were made at weekly intervals so that a relatively high resolution time-resolved pattern is available.

8.2.2.2 Catchment burden

Trees

The catchment burden of TCAA can be estimated with a high degree of certainty using the data of the routine sampling programme. The reservoirs of TCAA in the catchment are; needle material (various year classes), branch material, leaf litter and the soil. Several of these compartments were not considered by Hoekstra et al. (1999a) mainly because no measurements existed in the literature at that time. Branch and leaf litter were not regularly sampled at Dunslair Heights, but some determinations were made which are included in this model. The results for stem analysis were shown in Figure 7.4, but the non-routine data are included here as model parameters. Although the concentration of branch material is, when the total mass of branches is considered it may be a significant reservoir of TCAA. The TCAA concentration varied from 9 ng (g dwt\(^{-1}\)) TCAA in C+5 branches to 22 ng (g dwt\(^{-1}\)) TCAA in current year branches at Dunslair Heights. For use in this model a mean concentration of 16 ng (g dwt\(^{-1}\)) TCAA for all branch material has been assumed. It is not possible to assess the applicability of this value as these measurements have not been repeated, but excellent reproducibility of the triplicate analyses and similar concentrations in branches at Venlaw suggest that the concentration is likely to be close to this value.

The leaf litter from Dunslair Heights was analysed on three occasions using the same method as for needles (see Chapter 4). The results were previously reported in Table 5.1. The material was sampled from under tree stand A (Figure 5.1) at Dunslair Heights on 12/1/00, 14/8/00 and 1/11/00. The leaf litter has accumulated and compacted over a long period to form a layer of dead needles which is 5-10 cm thick lying above the top soil. During routine soil sampling it was removed before taking
the sample. Three separate sampling dates and locations were chosen to give a reasonable idea of the temporal and spatial variability of TCAA concentrations, but Table 5.1 shows that there is very little difference in concentration between the samples. This is surprising as faster TCAA degradation was expected in the summer months leading to lower concentrations. Since this was not observed, it suggests that leaf litter could be a considerable reservoir of TCAA. The heterogeneity expected in both the TCAA concentrations and the thickness of the litter layer in a forest stand is great, but it is assumed that the measured concentrations are representative of needle litter simply because of the precision of the results.

Although the concentrations of TCAA in the different compartments of the forest have been measured the other parameters that are required are estimates for density of planting and needle mass per tree at Dunslair Heights. For this information determinations of needle and stem dry weights of clones of Sitka spruce reported by Cannell et al. (1983) were used. Estimates of tree parameters were made after growing clones of Sitka spruce for 8 years followed by the destructive harvest of the trees and measurements of the dry weight of the different components. Parameters such as stem, branch wood, and needle mass per tree were made. One assumption that has been made for these calculations is that these 8 year old clones give a good estimate of the Sitka spruce at Dunslair Heights. Another parameter determined by Cannell (1982) was the percentage of total needles in each year class for Sitka spruce aged 30 years (Table 8.1). One parameter directly measured at Dunslair Heights is the planting density (distance between trees), which has been used to estimate the trees per unit area. The tree parameters are shown in Table 8.1 and 8.2.

<table>
<thead>
<tr>
<th>Needle age (years)</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>3-7</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 8.1; Distribution of needles by age for 30 year Sitka spruce trees
(Cannell 1982)
<table>
<thead>
<tr>
<th>Tree spacing</th>
<th>1.5 metres</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem dry wt</td>
<td>2.87 kg tree⁻¹</td>
</tr>
<tr>
<td>Branch wood dry wt</td>
<td>2.3 kg tree⁻¹</td>
</tr>
<tr>
<td>Needle dry wt</td>
<td>2.59 kg tree⁻¹</td>
</tr>
<tr>
<td>Projected needle area/tree</td>
<td>10.9 m² tree⁻¹</td>
</tr>
</tbody>
</table>

**Table 8.2; Sitka spruce tree parameters (from Cannell et al. 1983)**

(¹experimentally determined)

These parameters were determined from 8-year-old Sitka spruce and may not necessarily be applicable to the trees at Dunslair Heights, which are 46 years old. The needle mass per tree used by Juuti (1997) was 1.1 kg m⁻² dry weight, which is in good agreement with the reported mass of needles on 8 year old trees of 1.15 kg m⁻². The reason for this agreement is that in dense tree stands needles from lower branches are lost and so the bulk of the needles are situated in the crown of the trees. Cannell (*Pers. comm.*) estimated that 30 year old Sitka spruce contained 15-20 t ha⁻¹ dry weight needles based on many estimates from tree stands (Cannell 1982). This estimate gives 3.4 -4.5 kg (needles dwt) tree⁻¹, which is higher than in Table 8.2, but probably more representative of Dunslair Heights. This estimate can be further checked using the leaf area index for mature Sitka spruce (approximately 8 m² m⁻²). For each tree there is 18 m² of leaf area using the planting density (2.5 m² tree⁻¹). The ratio of dry needle mass to projected needle area per tree (0.24; from Table 8.2) is used to produce an estimate of 4.3 kg (needles dwt) tree⁻¹, which is in good agreement with the value estimated by Cannell. Therefore the value of 4.3 kg (needles dwt) tree⁻¹ is used for the mass balance calculations in this work.

The branch and stem mass of trees in a 46 year old stand is significantly greater than the 8 year old clones and so estimates must be made about the additional contribution. An estimate of the height of the trees at Dunslair Heights is 7 m with stem diameter (diameter at breast height) estimated at 30 cm. The volume of the stem can be estimated by assuming the stem to be a cone. Using Equation 7.1 in Section 7.4.5.1, the stem volume of a tree with these dimensions is calculated as 0.16 m³. The density of wood is estimated to be 0.45 kg l⁻¹ (fwt) or 0.3 kg l⁻¹ (dwt), from
which is estimated a mass of stem wood of 100 kg tree\(^{-1}\) (dwt). For a ratio of branch wood to stem wood of 0.8 is obtained an estimate of 80 kg (branch wood dwt) tree\(^{-1}\).

The determination of the absolute mass of tree components allows an estimate of the TCAA burden in trees at Dunsclair Heights using the needle TCAA concentrations measured in Chapter 4. The mean TCAA concentration of needles aged 1, 2 and ≥ 3 years over the whole of the 1999 growing season has been used to estimate the burden for that period. Since the combined mass of needles aged 3 to 7 years was calculated by Cannell \textit{et al.} (1983), this approach was used in the mass balance. The estimated masses and TCAA burden of needles are shown in Table 8.3.

<table>
<thead>
<tr>
<th>Year class</th>
<th>Needle mass tree(^{-1}) (kg)</th>
<th>Mean TCAA conc. ng (g dwt.)(^{-1})</th>
<th>TCAA burden (µg m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.3</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>3-7</td>
<td>2.1</td>
<td>59</td>
<td>123</td>
</tr>
<tr>
<td>Total</td>
<td>4.3</td>
<td>-</td>
<td>187</td>
</tr>
</tbody>
</table>

Table 8.3; TCAA burden in Sitka spruce at Dunsclair Heights

A similar calculation of burden is performed for the branch material. Combination of the average TCAA concentration in branches at Dunsclair Heights and the mass of branch wood per tree (80 kg m\(^{-2}\)) gives the burden of TCAA as 1300 µg m\(^{-2}\). The determination of the reservoir of TCAA in stems is more difficult as no measurement of TCAA concentration in stem material exists in the literature. The measurements in branch material at Dunsclair Heights have shown that TCAA concentrations decrease with increasing age so it is likely that 46 year old wood contains very small TCAA concentrations. It is likely that stems contain 0-5 ng (g dwt\(^{-1}\) TCAA resulting in a burden of 0 –1000 µg m\(^{-2}\) in stem wood. The uncertainty in this term has a large effect on the overall magnitude of TCAA in the tree reservoir. Another effect of the tree stands on the mass balance is the contribution of TCAA present in leaf litter, which is considered later in the soil estimates.
Soil

The most important, yet the least well characterised compartment is the soil. The reasons for this are the huge variation of TCAA within soils, and also the lack of reliable measurements in the literature. Measurements of soil density at Dunsinair Heights have been performed on as sampled soils using a displacement method (Wild 1993). The dry bulk density determined on 3 samples was 0.38 g cm\(^{-3}\) (Table 8.4). This was slightly higher than expected since the top layer of soil is almost a peat and was expected to have a dry bulk density in the range 0.1 to 0.3 g cm\(^{-3}\). For the purposes of this model the soil layer is assumed to be 0.5 m deep, so using the density measurements above, the total mass of soil is calculated as 190 kg m\(^{-2}\).

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>Soil mass (g fw)</th>
<th>% Water</th>
<th>Soil mass (g dw)</th>
<th>Volume (cm(^{3}))</th>
<th>Dry bulk density (g cm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>S05</td>
<td>23.53</td>
<td>53.3</td>
<td>10.99</td>
<td>20</td>
<td>0.55</td>
</tr>
<tr>
<td>S03</td>
<td>33.48</td>
<td>65.4</td>
<td>11.58</td>
<td>34</td>
<td>0.34</td>
</tr>
<tr>
<td>S06</td>
<td>22.33</td>
<td>71.4</td>
<td>6.39</td>
<td>26</td>
<td>0.25</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.38 ± 0.15</td>
</tr>
</tbody>
</table>

Table 8.4; Soil density measurements from Dunsinair Heights

\(fw = \text{fresh weight}; \ dw = \text{dry weight}\)

The most important decision to take in this mass balance is the most appropriate soil TCAA concentration to use in the calculation. The soil data has been divided into samples from each type of soil i.e. tree stands, rides and open moor (Chapter 5). The average TCAA concentrations of each soil type are shown in Table 8.5.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Mean TCAA ng (g fw)(^{1})</th>
<th>Mean TCAA ng (g dw)(^{1})</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree stands (high values only)</td>
<td>217 ± 116</td>
<td>524 ± 238</td>
<td>6</td>
</tr>
<tr>
<td>Tree stands (except high values)</td>
<td>16 ± 22</td>
<td>51 ± 61</td>
<td>13</td>
</tr>
<tr>
<td>Tree stands (all values)</td>
<td>76 ± 116</td>
<td>190 ± 265</td>
<td>19</td>
</tr>
<tr>
<td>All samples</td>
<td>37 ± 81</td>
<td>92 ± 190</td>
<td>45</td>
</tr>
<tr>
<td>All samples (except tree stands)</td>
<td>9 ± 5</td>
<td>20 ± 10</td>
<td>26</td>
</tr>
</tbody>
</table>

Table 8.5; Mean soil TCAA concentrations at Glentress Forest

\(\text{Error ranges are standard deviations of 'n' number of sites.} \quad \text{Each site is a mean of triplicate analyses.} \quad fw = \text{fresh weight}; \ dw = \text{dry weight}\)
As previously discussed in Chapter 5 not all the concentrations in soils under tree stands are high, but the high values only occur under tree stands. Therefore, it would be wrong to use the high or the low mean soil TCAA concentrations, as this would not be truly representative. Therefore the mean of all the tree stand measurements of soil TCAA concentration is used in the calculations. For a comparison, the mean of all samples (except those in tree stands) is considered to provide an estimate of a background soil concentration. The soil TCAA burden is shown in Table 8.6.

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>Soil mass kg m(^{-2}) (dw)</th>
<th>Mean TCAA ng (g dw(^{-1}))</th>
<th>Soil TCAA burden (\mu g) m(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree stands</td>
<td>190</td>
<td>190</td>
<td>36100</td>
</tr>
<tr>
<td>Non-tree stands</td>
<td>190</td>
<td>20</td>
<td>3800</td>
</tr>
</tbody>
</table>

Table 8.6; Soil TCAA burden at Dunslair Heights

The final component of TCAA in the catchment is present in the needle litter layer. At Dunslair Heights this is very prevalent with a thickness of approximately 8 cm. Similar measurements of density by displacement have been performed on this layer as outlined previously for soil and give a density for the leaf litter of 0.21 g cm\(^{-3}\) (see Table 8.7).

<table>
<thead>
<tr>
<th>Litter ID</th>
<th>Litter mass (g fw)</th>
<th>% Water</th>
<th>Litter mass (g dw)</th>
<th>Volume (cm(^3))</th>
<th>Dry bulk density (g cm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>1) 21.24 2) 30.84 3) 29.96</td>
<td>79.0</td>
<td>1) 4.46 2) 6.48 3) 6.29</td>
<td>1) 22 2) 32 3) 30</td>
<td>1) 0.20 2) 0.20 3) 0.21</td>
</tr>
<tr>
<td>S02</td>
<td>1) 29.52 2) 24.64 3) 27.05</td>
<td>76.6</td>
<td>1) 6.91 2) 5.77 3) 6.33</td>
<td>1) 30 2) 26 3) 29</td>
<td>1) 0.23 2) 0.22 3) 0.22</td>
</tr>
</tbody>
</table>

Table 8.7; Density measurements of needle litter at Dunslair Heights

(Each sample density measured in triplicate)

Using the density and thickness measurements the mass of the needle litter layer is calculated to contain 16.8 kg m\(^{-2}\) (dry weight). Using the mean of the measured TCAA concentrations in needle litter of 160 ng (g dw\(^{-1}\)) TCAA (Table 5.1), the burden of TCAA is calculated to be 2700 \(\mu g\) m\(^{-2}\).
8.2.2.3 Output fluxes

The final task is to calculate the output fluxes for the model. This is one of the hardest tasks as the parameters needed to do this are few and far between in the literature and a good understanding is required of the hydrogeology of the site. Some estimates can be made from the available measurements. The output flux may be represented as follows:

\[
F_{\text{output}} = F_{\text{seepage}} \quad \text{Equation 8.3}
\]

where \( F_{\text{seepage}} \) is the TCAA taken into the water that passes into the soil, but ultimately makes its way to local surface water outflows i.e. streams, on a relatively fast time-scale. This parameter has not been routinely measured throughout the study at Glentress Forest, but an estimate can be made from some of the measured data. Extraction experiments performed on soil have shown that TCAA is not easily leached from soil by water. The TCAA measured in the water extracts of soil indicates the amount of TCAA that can be released into or is present in the pore water of the soil. The absolute amounts of leachable TCAA are not important, but the percentage of TCAA leachable from the total TCAA present in the soil is a useful parameter. The results are shown in Table 8.8.

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>TCAA in extracts ng (g fw)^{-1}</th>
<th>% of total TCAA</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>S02 (9/2)</td>
<td>11.2 ± 3.5</td>
<td>4.6</td>
<td>5</td>
</tr>
<tr>
<td>S05 (14/8)</td>
<td>15.7 ± 0.8</td>
<td>6.0</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 8.8: Leachable TCAA as % of total present in the soil

\( \text{(Mean ± standard deviation of } n \text{ soil extractions.} \) \n
\( \text{Each extraction represents triplicate analysis of extracts) } \)

If the assumption is made that the same percentage of TCAA is leached from every soil at Dunslair Heights then the outward seepage flux of TCAA can be estimated. The flux \( F_{\text{seepage}} \) would therefore be 5.3% of the total soil burden i.e. 1900 \( \mu \)g m\(^{-2}\) yr\(^{-1}\) for the tree stands or 200 \( \mu \)g m\(^{-2}\) yr\(^{-1}\) for the background soil.
This hypothesis may be tested using measurements from Glentress Forest. If the assumption is made that the percentage of leachable TCAA is completely removed from the system in the yearly rainfall at Dunslair Heights (1062 mm or 1 m²) then the concentration expected in the seepage would be 1.8 ppb TCAA. Measurements were made in stream/ outflow samples at Dunslair Heights to investigate whether the theoretical concentration was measured. At Dunslair Heights samples were taken in streams/ outflows originating from the main stand of trees studied. This was sampled at 3 elevations down the hillside to investigate any dilution of TCAA in the stream. The difference in elevation between the top and bottom sample was 100 m. Other samples were taken at Shieldgreen, where the streams from Dunslair Heights had merged to form a burn, and also in a small stream originating directly from the tree stands at Venlaw site. This provided a comparison between the two forested locations. The measured concentrations are shown in Table 8.9.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Concentration (ppb TCAA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH (Top)</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>DH (Middle)</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>DH (Bottom)</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Shieldgreen burn</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Venlaw</td>
<td>2.6 ± 0.2</td>
</tr>
</tbody>
</table>

Table 8.9; TCAA concentrations in streams and outflows

(Error ranges are standard deviations on triplicate analyses)

The concentration measured in Shieldgreen burn is in close agreement with the predicted concentration from the seepage flux. However, at a peaty site such as Dunslair Heights 100% of wet deposition will not be free to percolate through to groundwater so the concentration of TCAA seeping from sites with high soil concentrations such as Dunslair Heights may be greater. It is likely that only 70% of the wet deposition will reach the streams with the remaining 30% of the water either intercepted by foliage and then subsequently evaporated or taken up by the root system of the trees and transpired back to the atmosphere. This would lead to higher
concentrations than calculated. Assuming the leachable TCAA is taken into 70% of the yearly rainfall the concentration in outflow from the tree stand should be 2.6 ppb. This is in closer agreement with the measured concentrations in the upper stream samples at Dunslair Heights and the Venlaw outflow sample. These measurements suggest that there is a large contribution from forested soils to the total TCAA concentration in the streams. Even at Venlaw, where the stream only had to trickle 100 m through forest soil to join with the burn, there is an elevated concentration in the outflow from the forest. The forest measurements also validate the assumptions made in the mass balance calculations about the level of the seepage flux \( F_{\text{seepage}} \). More detailed studies could estimate this seepage flux using lysimeters or routine monitoring of the stream concentrations using flow proportional samplers.

8.3 Results and discussion

A schematic representation of the calculated mass balance model for Dunslair Heights is shown in Figure 8.2. It shows the calculated input and output fluxes and reservoirs of TCAA in a generalised tree stand at Dunslair Heights, as outlined in the previous section. The calculations show that the output flux from the model volume is 2-fold larger than the input flux. This scenario suggests that there is a net source of TCAA within the model volume. This difference can be attributed either solely to \textit{in situ} production of TCAA, or to a net imbalance between \textit{in situ} TCAA production and degradation. The time-integrated fluxes are much smaller than the total catchment burden of TCAA suggesting that TCAA cycling is dominated by internal catchment processes. It is difficult to distinguish between the competing processes in the system, but by using the measurements at Dunslair Heights a discussion about them can be attempted. The calculated values in the model are dominated by the large reservoir of TCAA in the soil, which is about 10-fold higher than for the other compartments. It is therefore likely that it is soil processes which determine the overall cycling of TCAA in the environment and which lead to the net increase in output flux. The TCAA reservoir in the trees is minor compared to the magnitude of the soil reservoir and only the leaf litter is likely to perturb the cycling of TCAA significantly. The processes possibly occurring in the model are discussed below.
Figure 8.2; Calculated mass balance model at Dunsclair Heights
The most probable scenario is that the net production of TCAA in the catchment is caused by the production of TCAA in the soil with little or no degradation. Measurements of rates of soil degradation reported in Chapter 5 have shown no evidence that TCAA is degraded significantly over 36 days, which suggests that the net production is not caused by an imbalance between significant degradation and production processes. The presence of a large soil reservoir (36 mg m\(^{-2}\)), which leads to the high leached concentrations in the outflow streams, means that any degradation flux has to be balanced by a huge production flux. This is possible as Hoekstra *et al.* (1999a) estimated the production flux in the soil top layer to be 60 ± 90 mg m\(^{-2}\) yr\(^{-1}\) in the top layer of spruce forest soils. The concentrations of TCAA are very heterogeneous in soil (Chapter 5), which are thought to be caused by variable production rates throughout the stands (and if present, variable degradation rates). The large soil reservoir is thought to have been caused by the accumulation of TCAA over time but, at this stage, it is not possible to distinguish whether this is an accumulation of naturally produced TCAA or TCAA from atmospheric input.

The second scenario is that, as TCAA is leached out of the catchment in the seepage flux, previously bound TCAA is mobilised into the soil solution. This scenario would result in a gradual decrease in the overall soil reservoir, which has not been measured at Dunslair Heights and seems unlikely due to the accumulation over time that has led to such a large soil TCAA reservoir.

The final scenario is that as TCAA is removed in seepage flux there is a flux of TCAA into the soil from the litter layer, which is maintained by the input of dead needles from the trees. The imbalance between the input and output fluxes have to be accounted for by this needle litter flux is approximately 1000 μg m\(^{-2}\) yr\(^{-1}\). As the concentration of TCAA in the needle litter layer has been demonstrated to be relatively constant the loss of TCAA to the soil must be balanced by the input to the litter layer of TCAA from fallen needles. The total reservoir of TCAA in needles is only 200 μg m\(^{-2}\), so the litter layer cannot account for the imbalance between input and output even if all the living needles were dropped and the TCAA present passed into the soil. These calculations suggest that there is a (net) natural production of
TCAA in the soil, but these scenarios are based on the accurate determination of the fluxes and reservoirs in this model. The uncertainties in the calculations are discussed below.

The calculated output flux in stream outflows at Duns Blair Heights is crucial to the conclusions drawn from this box model, but it has only been directly measured in a few samples (<10). The output flux calculated in this model for Duns Blair Heights and by Hoekstra et al. (1999a) is the greatest source of uncertainty in the calculations. The latter author calculated output flux using the concentration in pore water and the rain volume, assuming that all the TCAA would completely leave the system. There are limited measurements of pore water concentrations in the literature and there is uncertainty in this approach.

Measurements of TCAA in stream water and outflows from the tree stands at Duns Blair Heights have shown an elevated concentration of TCAA compared with the concentration in direct atmospheric deposition. The mean TCAA concentration in rain of 0.84 ppb is far exceeded by the concentration in direct outflows from tree stands at Duns Blair Heights (3 ppb) and at Venlaw (2.6 ppb). The concentrations in the streams, which contained seepage from all types of site in the forest (1.5 ppb) are also higher than the rain concentrations. This evidence alone suggests that there is a net contribution of TCAA in the catchment. At Duns Blair Heights assumptions have been made about the percentage of TCAA leachable from the soil based on the extraction experiments in Section 5.5.4. It is possible that the percentage of extractable TCAA may vary according to the soil type, as it is likely to be determined by the humic acid/organic matter content and the capacity of the soil to bind TCAA. However, no simple relationship between total organic matter content and TCAA concentration exists (Section 5.5.6) and it is likely that the humic and fulvic acids in the soil determine binding. Although the seepage flux ($F_{seepage}$) that has been used here is likely to be a good estimate, further work using lysimeters can determine the kinetics of soil binding to provide a more precise value. The one-year integrated value of $F_{seepage}$ is small compared with the whole reservoir of soil TCAA, but in this model accounts for the removal of 5% of the soil burden. In the previous
section the concentration calculated in the outflows from tree stands at Dunslair Heights (2.6 ppb) is in excellent agreement with the measured concentrations (Table 8.9), which further supports the accuracy of the calculated output flux.

There is more confidence in the accuracy of the calculated input flux of TCAA at Dunslair Heights because of the large number of measurements made (>200). The input of TCAA to forest via wet deposition is approximately 1000 µg m\(^{-2}\) yr\(^{-1}\) at Dunslair Heights, which is higher than measured in Switzerland (110-150 µg m\(^{-2}\) yr\(^{-1}\)) (Reimann, 1996) and Finland (50 – 100 µg m\(^{-2}\) yr\(^{-1}\)) (Juuti, 1997). However, it is not certain whether the European deposition is calculated for a forest or open ground, whether it included wet deposition from clouds and the elevation of the site studied.

The wet deposition flux in rain used in this model (890 µg m\(^{-2}\) yr\(^{-1}\)) is about 40% larger than that of Hoekstra et al. (1999a) (600 µg m\(^{-2}\)). This arises from higher measured TCAA concentrations than used by Hoekstra rather than a higher rainfall rate. The mean concentration of TCAA in rain during the period April 1999 to April 2000 was 0.84 ppb compared with that of 0.2 ppb used by Hoekstra et al. (1999a). The additional contribution from cloud input of TCAA at Dunslair Heights (125 µg m\(^{-2}\) yr\(^{-1}\)) was not incorporated by Hoekstra et al. (1999a) in their calculations. Few measurements of cloud TCAA have been reported in the literature, but it is an important contribution if an upland forested site is to be considered as the concentration of many major ions have been reported to be enhanced in cloud compared with rain (Crossley et al. 1992, 1998). This also causes the wet deposition flux to be higher in this mass balance model than by Hoekstra et al. (1999a). The atmospheric flux is calculated from actual measurements at the Dunslair Heights site and so uncertainty exists only in the accuracy and precision of the triplicate analytical measurements.

The dry deposition of TCAA calculated by Hoekstra et al. (1999a) of 50 µg m\(^{-2}\) yr\(^{-1}\) for a spruce forest appears overestimated, because the value of 0.1-1 pg l\(^{-1}\) chosen for a typical air concentration is high. The minimum value of 0.1 pg l\(^{-1}\) (100 pg m\(^{-3}\)) used by Hoekstra et al. (1999a) is equal to the maximum determined at Dunslair Heights, where the mean TCAA in air concentration was only 30 pg m\(^{-3}\). It was also
assumed by Hoekstra et al. (1999a) from the literature measurements that the air concentrations in a forest were higher than in an urban environment, an assumption based on a limited set of measurements. The results from 1 year of sampling at Edinburgh show that the concentration in the city (mean: 95 pg m$^{-3}$) is higher than in Glentress Forest (mean: 30 pg m$^{-3}$). This could be because higher local concentrations of chlorinated solvent precursors in cities lead to higher local TCAA concentrations, but this seems unlikely as the proposed reaction to form TCAA is slow and higher local concentrations of the precursors will quickly become well mixed in the turbulent boundary layer. Alternatively TCAA may be formed from power generation, transport and incineration in urban environments. No explanation exists in the literature for the supposed higher concentrations in rural sites. It does not seem logical that TCAA should be in higher concentrations in forest air, especially as no volatilisation of TCAA is possible from the soil or wet deposition. The uncertainty in the input flux calculated for dry deposition at Dunslair Heights (10 µg m$^{-2}$ yr$^{-1}$) arises only from using an inappropriate deposition velocity rather than an incorrect air concentration. However, the contribution of dry deposition to the total atmospheric input is small and any uncertainties in its calculation do not alter the findings of the box model calculations. Further measurements of air concentrations would be interesting to calculate the spatial variability of TCAA from site to site or from urban to rural location.

Certain omissions must be discussed here with respect to the model calculations. No loss of TCAA from the model has been described with respect to volatilisation. The high solubility (Worthing & Hance 1991) and Henry's constant (Bowden et al. 1998) of TCAA means that TCAA formed in the soil, or accumulated from wet deposition does not partition into the atmosphere. The other possible source of TCAA to the catchment, which has not been suggested, is the direct formation of TCAA from its C$_2$-precursors in the soil. The main source of TCAA in the soil has always been assumed to be by the chlorination of organic matter by the action of chloroperoxidase enzymes on humic material using chloride (Hoekstra et al. 1995a, 1998b). Another suggestion has been the input of these chlorinated solvents and their transformation by photolysis in an analogous process to its formation in the atmosphere. However,
solar radiation is unable to penetrate the soil, especially in a forest, so conversion of C₂-precursors to TCAA is unlikely. This process has not been considered in this study as no evidence of TCAA formation in soil from atmospheric C₂-halocarbons has been presented in the literature to support it, but it should be borne in mind as a possible source of uncertainty at the soil surface.

The largest and most important reservoir in the model is the soil, which impacts the most on TCAA, whether by storage, production or degradation. It is the main conduit by which trees take up TCAA, but is also a buffer that protects trees from more extreme exposure. Whether the huge reservoir of TCAA is present in the soil after formation \textit{in situ} or accumulated by sorption or binding from wet deposition is uncertain. The soil appears to bind the TCAA meaning it cannot be leached into the soil pore water, thereby preventing TCAA from being taken up by the root system of the trees. This binding is probably the process that inhibits huge concentrations from entering the trees and from possibly overloading their metabolism. However, there is a clear accumulation process that has produced the huge soil reservoir, which is unlikely to be reduced significantly over time. The accurate measurement of the size of the soil reservoir and the characterisation of its internal processes remains the least well understood facet of the biogeochemical cycling of TCAA. Despite the large TCAA concentrations in soil and their heterogeneity, the variation of TCAA in current year needles over a growing season suggests that trees are capable of maintaining a concentration that is not harmful to themselves at these environmental conditions by metabolism. The dropping of older needles with normally higher concentrations may explain the high concentration of TCAA in the litter layer.

It has long been assumed (Frank \textit{et al.} 1991; Haiber \textit{et al.} 1996) that the TCAA bound to the humic material can be considered degraded or lost. Therefore in the determination of TCAA degradation rates in the literature it is the loss of measurable TCAA that has been determined. The derivatisation methodology that is used to determine TCAA in soil utilises an extraction step that has been shown to be inefficient (Section 5.5.4). Frank (\textit{Pers. comm.}) suggested that since TCAA is used as a precipitating agent in protein technology, it presumably binds to organic matter
through its $-\text{NH}_2$ functional groups. However, no suggestions have been made by these workers to improve the extraction of TCAA from soil. More accurate measurements of soil TCAA concentrations may lead to an increase in the suggested half life, since TCAA that was previously thought to be degraded may be proved to have been bound to the soil.

The degradation flux of TCAA in the soil is the most difficult parameter to assess as it has not been experimentally derived at environmental concentrations. Frank et al. (1991) suggested a degradation rate of 7% day$^{-1}$, which corresponded to a half life of 10 days. If, as Haiber et al. (1996) suggested, 90% of TCAA in solution becomes bound or degraded within 2 hours then the calculated half life of TCAA in soil is very short. Half life measurements in the literature of 15 to 90 days (Worthing & Hance 1991), 14 to 90 days (Martin 1972) and 10 to 68 days (Torstensson & Hammarström 1981) would suggest that the half life is not as short as the 50 days predicted by Hoekstra et al. (1999a), and that one value for the half life of TCAA does not exist for all soils and conditions. More recent work by Matucha et al. (2000b) has estimated half life in soil using $^{14}$C-labelled TCAA. Two separate laboratory experiments showed a degradation rate of 1.1 % day$^{-1}$ ($t_{1/2}$ 66 days) and 3.7 % day$^{-1}$ ($t_{1/2}$ 18.4 days). No consensus exists in the literature as to the half life of TCAA and it seems that a half life of 50 days as used by Hoekstra et al. (1999a) is not appropriate at Dunslair Heights. Therefore it is likely that half lives determined using derivatisation methodology are influenced by the adsorption of TCAA to soil. Schölzer (Pers. comm.) reported the degradation rate to be 0.1% day$^{-1}$ corresponding to a half life of 700 days which, though much longer than the reported values, may be more appropriate to Dunslair Heights. Schölzer also suggested that the degradation rate is determined by the organic matter content of the soil. The key point is that it is very important that a differentiation between degradation and binding is made. It is particularly important that TCAA analytical methods are able to clearly distinguish between TCAA that is extractable and that which remains bound within the soil matrix (see Chapter 5).
The estimate of the contribution of potential degradation of TCAA in soil to the system is therefore another weak point of mass balance models. The degradation rates are assumed here to be close to zero, which are representative of the soil in the study at Dunslair Heights. Work in this study on the stability of TCAA in soil has shown that TCAA does not significantly degrade over 36 days at 4°C (see Section 5.5.5). What is certain is that the overall size of the soil reservoir means that the production rate must be far in excess of any degradation rates. Hoekstra et al. (1999a) used the calculation in Equation 8.4 to estimate the degradation flux in the top layer of soil ($D_{soil}$) as follows:

$$D_{soil} = 365 \ln \frac{2 \cdot C_s \rho_s d}{t_{1/2}}$$

where $C_s$ is the soil TCAA concentration [ng (g dwt)$^{-1}$], $\rho_s$ is the bulk dry soil density (g m$^{-3}$), $d$ is the thickness of the surface layer where degradation occurs (m) and $t_{1/2}$ is the half life of TCAA in soil (days). Hoekstra et al. (1999a) suggested that a suitable value for $d$ was 0.1 metres, which if used with the measurements reported from Dunslair Heights and a half life of 50 days gives a degradation flux at Dunslair Heights for the tree stands of 37000 µg m$^{-2}$ yr$^{-1}$. The calculated degradation flux ($D_{soil}$) is large and would be the major removal process for TCAA in the system. The implication of this calculation is that there is only degradation in the top layer of soil and does not describe the processes occurring in the soil layer as a whole. This finding would suggest a huge production flux was needed in the top layer to maintain the concentrations detected. A higher degradation rate at the soil surface would explain some of the findings of the vertical profiles (Section 5.5.3), which showed peak concentrations at 15 - 22.5 cm depth in one 30 cm soil core. The half life of TCAA in soil is influenced greatly by the specific soil conditions and therefore much research on the half life of TCAA in different soil types at environmental concentrations is needed to verify mass balance calculations. The heterogeneity of soil in tree stands may lead to variations in both half life and soil density vertically and horizontally, so appropriate degradation rate measurements must be assessed on each soil type.
The removal processes are key to understanding the soil reservoir as these will help to maintain the soil TCAA concentration and therefore the total reservoir of TCAA and enable an estimate of the soil production rate to be made. The effect of different half life values of TCAA in soil is compared (Table 8.10) using literature estimates of 10 days (Frank et al. 1991), 50 days (Hoekstra et al. 1999a) and 99 days (Schöler). The implications are vastly different for the soil compartment depending on the half life used. The degradation flux calculated using Equation 8.4 (Hoekstra et al. 1999a) at each TCAA half life is shown.

<table>
<thead>
<tr>
<th>( t_{1/2} ) (days)</th>
<th>Calculated ( D_{\text{soil}} ) (mg m(^{-2}) yr(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>183</td>
<td>Frank et al. 1991 (maximum ( t_{1/2} ))</td>
</tr>
<tr>
<td>50</td>
<td>37</td>
<td>Hoekstra et al. 1999a</td>
</tr>
<tr>
<td>99</td>
<td>18</td>
<td>Schöler [Pers. Comm.] (minimum ( t_{1/2} ))</td>
</tr>
</tbody>
</table>

Table 8.10: The effect of the half life of TCAA in soil on degradation flux (\( D_{\text{soil}} \))

With a half life for TCAA of 10 days the soil reservoir will be degraded rapidly and the overall cycling of TCAA will be fast in the system whereas if the longest half life is used then the degradation flux will be an order of magnitude smaller. The soil TCAA production rate required to maintain the high concentrations measured under the tree stands would also vary significantly. Measurements of the degradation rate of TCAA in soil made in this study after storage at 4°C has suggested that there is no significant decrease in TCAA concentration over 36 days. This suggests that none of the half life estimates for degradation (Table 8.10) are appropriate for the mass balance calculations at this site. An explanation for this stability may be due to the location of TCAA within the soil. It is possible that unbound TCAA in the soil solution may be degraded, but TCAA adsorbed to the soil may not. The leaching experiments in Section 5.5.4 suggested that 95% of TCAA is bound in the soil and so this would explain why degradation of TCAA in the soil is hard to detect. An experiment to test this would be to sample a soil and centrifuge a sub-sample to remove the soil solution. The concentration of TCAA in the soil and soil solution would be determined and then after a period of storage another sub-sample could be
analysed in the same way. The degradation rate would then be determined in the whole soil and in the individual components of the soil. This is one possible explanation why the half life of TCAA in soil from Dunslair Heights is probably much longer than in the literature.

The final discussion of the model should concern the reservoirs. The soil and vegetation reservoirs of TCAA in the model are much larger than the input and output fluxes in the catchment. The soil TCAA, as discussed above, may be degraded by elimination processes, but TCAA present in the trees and the litter layer remain and must be considered. The TCAA calculated to reside within trees (approximately 2.5 mg m\(^{-2}\)) is smaller than in the soil. The uncertainties about stem and branch concentrations, discussed previously, mean that this value may vary from 0.2 - 4 mg m\(^{-2}\), which is a 20 fold difference. Stem and branches make up 98% of the mass of the trees and so the presence of only a small TCAA concentration would significantly increase the total TCAA reservoir. The assumptions about branch and stem TCAA concentrations cannot be critically assessed, as limited information is available. The first measurements of stem TCAA concentrations in the literature were reported in Chapter 7, made on stems from Dunslair Heights and Venlaw at a single sampling date. The concentrations measured were small [9-22 ng (g dw)\(^{-1}\) TCAA] and demonstrated a trend of decreasing TCAA with year class. These stems required homogenisation prior to analysis and so mostly the thinner stems were analysed. The main branch material is likely to be thicker and denser so it is not known how representative these measured concentrations are of all branch material. It is likely that as branches become older and denser with lower water content the TCAA concentration may decrease to zero. The TCAA concentration of stem material is totally uncharacterised as no measurements exist. In common with branches, the concentrations of TCAA in stems are likely to be close to zero, but any non-zero concentration contributes greatly to the tree reservoir. These two components have been previously ignored because of the inability of the derivatisation methodology to determine TCAA in stems. If a representative concentration for a tree, a stand or a forest could be determined then the whole mass balance would be better characterised. Matucha et al. (2000a) reported work
investigating uptake of TCAA by Norway spruce by the application of isotopically labelled TCAA to seedlings. This study showed that concentrations of TCAA were detected in both branches and stems with concentrations 50% and 25% of the needle concentrations respectively. However, the results from seedling experiments are very different to those in mature trees, which are likely to be denser, with more bark and therefore less TCAA. Furthermore the TCAA concentrations used by Matucha et al. (2000a) are not representative of the environmental exposure, although the study does illustrate that TCAA may be stored in these tree components.

In contrast, there is considerable certainty in the TCAA concentrations of the needle material following the extensive temporal measurements undertaken at Dunslair Heights. The calculations of Juuti (1997) assumed that a coniferous forest had a mass of needles of 1.8 kg m\(^{-2}\) on a fresh weight basis. Correction of this value for needle water content (assumed 40%) gives a value of 1.1 kg m\(^{-2}\), which is lower than that assumed at Dunslair Heights. The effect of an overestimate of the vegetation reservoir on the mass balance is lessened by the presence of the large soil reservoir and so is unlikely to affect the outcome of the calculation. A final effect of the presence of TCAA in the non-photosynthetic parts of trees is that it may effectively remove TCAA from the cycle, as this TCAA is unlikely to be degraded or recycled to the soil like that in older needles. This means that branch and stems may be an effective sink of TCAA.

The final reservoir of TCAA is the needle litter layer above the soil. An estimate of 190 ng (g dwt)\(^{-1}\) TCAA was used as this was the concentration measured both in winter and summer at Dunslair Heights. Although degradation was assumed to occur the concentration has remained the same over a whole growing season, suggesting that TCAA is at steady state in the litter layer. The litter layer at this site has accumulated over several decades. Many of the younger stands do not possess this litter layer because they have not become so limited for space and light causing most of the needles on the lower branches to be dropped. It is possible that the thick litter layer is one of the causes of the high TCAA concentrations in soil, whereby the top layer of soil is insulated from the air possibly leading to the net build up of TCAA.
Whether this is because of a greater production rate or just a lower degradation rate is uncertain. More research on the role of the litter layer, which is much ignored in the literature, is essential.

One further degradation flux that has not been considered is the possibility of degradation of TCAA in the needle litter layer or transfer from the litter to the soil. The transfer is not significant as the combined soil and litter burden will be constant, however if there is degradation its characterisation would be a useful addition to the model. It is assumed that there is no degradation as the measurements show that the litter concentration has remained constant over the year (Table 5.1). This could also be explained by a constant input of recently dropped needles or wet input to the litter meaning that the layer is effectively in a steady state between input and degradation/output. There are not enough data to distinguish between these hypotheses in this study.

The process of degradation is the most efficient method of removing TCAA from the system, but it is not known to what extent it occurs at Dunsclair Heights. Uncertainty about the formation rate of TCAA in the soil is matched by the uncertainty about its metabolism therein. The heterogeneity of the TCAA concentrations that have been measured within the soil has been thought of in terms of differing rates of production, but this model suggests that it could also be due to varying net rates of production arising from differing degradation rates. There is no understanding of the processes that cause TCAA to be formed, bound or degraded in the soil, but if future studies are to investigate this it should be at a small spatial scale. The understanding of the kinetics of binding and formation at true environmental concentrations is paramount.

8.4 Conclusions

The conclusions from this mass balance study are affected by the degree of certainty with which input and output fluxes are known. The comparison of concentrations in the input and output measurements suggest that there is an increase of TCAA in the catchment, which suggests a net production source. The main discussion in this field
of research is focussed on the correct measurement and interpretation of soil TCAA concentrations. The concluding remarks at the Atmospheric Reactive Substances Conference (Bayreuth, 1999) stated that the soil matrix should be investigated more thoroughly, and was identified as the main area where measurements and research was lacking. This model has shown that the soil compartment is the dominant part of the model, and that slight changes in the assumed half life and soil concentrations used significantly alter the results and the conclusions drawn. However, it is not possible from these measurements to differentiate between the processes of production and degradation that occur and produce the large soil reservoir. The net production of TCAA in the catchment is tentatively suggested to be of a natural origin as no anthropogenic sources can explain it, but this should be studied further possibly with isotopically labelled studies.

The precision of the measurements of TCAA in wet deposition and needles is good with uncertainties limited to the analytical precision of the method. Measurements have been made throughout the growing season so that short term variations have been accounted for, and the absolute atmospheric input and reservoir of TCAA in needles have been determined. The uncertainty exists in the contribution of branch and stem material to the reservoir of TCAA in trees. These factors are of minor importance if the soil TCAA cannot be accurately characterised. The maximum burden of TCAA in trees is only 10% of the TCAA in the soil so research must be focused on making accurate measurements of soil TCAA concentrations and subsequent half life determinations in order to estimate the TCAA production rate.

The integrated external fluxes are clearly much smaller than the total catchment burden, which points to the scenario that TCAA cycling is likely to be dominated by internal catchment processes, of which the dominant process is the production and degradation flux of TCAA. There is large uncertainty in this flux, as the density of the soil and the half life of TCAA which determines it, is not well characterised in either this study or the literature. This process should be characterised in terms of different soil compositions especially those of soils found with high TCAA concentrations. The dominance of the soil means, in the opinion of this author, that
the majority of research activity should be focused on this compartment. Clear problems exist in the analysis of soil, but these should be challenged in order to improve the understanding of the TCAA cycle.

The obvious wider conclusion from this model is that the atmospheric formation and input of TCAA from possible chlorinated precursors make a minor contribution to the overall reservoir of TCAA. The input of atmospheric TCAA is only a minor perturbation to the large soil reservoir and is equivalent to the annual net production of TCAA measured in the output flux. Whether there is natural production of TCAA, which remains in the catchment can only be assessed over time by monitoring the increase in the soil TCAA reservoir. This probably means that the industrial production of TCAA is smaller than the TCAA from natural production in the soil. It has always been assumed that some natural formation of TCAA must exist in order to explain its presence in samples from before the industrial revolution (von Sydow et al. 1999 & 2000), but it was not known in what matrices this natural formation would occur. The main conclusions of this model are to show the direction in which research should be heading, and to agree with the findings of Hoekstra et al. (1999a) that evidence exists for a natural formation of TCAA in soil.
Chapter 9

Conclusions and future work

9.1 Analytical development

During the development of the methodologies for the analysis of TCAA three approaches have been shown to work well with all matrices of interest. Two labour-intensive approaches, namely the Modified Nielson-Kryger steam (MoNKS) distillation and the manual Headspace Gas-Chromatography (HSGC), have been established and have produced reliable, robust methods. Both approaches use inexpensive equipment and are simple to operate. However, routine sampling and analysis that requires a large throughput of samples to investigate patterns ideally requires an automated technique, which places the emphasis on the interpretation of results rather than the time-consuming manual injection of samples. The automated technique developed in this study has been shown to reliably analyse samples from matrices as diverse as rain and soil with detection limits in the ng g\(^{-1}\) range. The direct analysis of samples without the need for extraction is an added advantage of HSGC. The ability to use an increased sample mass or volume enables a lower detection limit to be achieved rather than to require a pre-concentration step. The use of solid-phase extraction (von Sydow \textit{et al.} 1999) for the analysis of TCAA in aqueous samples is easy, but the elution step may not give full recovery, and could also lead to contamination from the reagents used.

The numerous derivatisation methods employed by the majority of other workers assume a facile extraction of TCAA from the matrix into water with shaking. As has been proved in Chapter 2 the analysis of whole needles yields larger concentrations than frozen ground needles, which suggests that TCAA may not be easily extractable from needles using water alone. Previous comparisons between the diazomethane derivatisation method and HSGC (Frank \textit{et al.} 1990) used ground needles for the derivatisation method and whole needles for HSGC. The comparison in results from
the two methods was good, but may have been improved if like were compared with like.

Soil has proved to be the most difficult matrix in which to determine TCAA concentrations. The lack of measurements of TCAA in soil in the literature has hinted at the difficulties that are inherent with soil analysis. Hoekstra (Pers. comm.) has reported the problems encountered with the recovery of TCAA during soil spiking experiments. Other work by Haiber et al. (1996) has suggested that TCAA can be adsorbed onto humic material present in solution giving apparent evidence of aqueous phase degradation. If irreversible adsorption is occurring this is further evidence for the inability of the derivatisation techniques to determine all the TCAA present in soil. Soil analysis results presented by several authors at the Haloacetic Acids Conference, Toronto (2000) indicated that analysis by each of the two types of methodologies (derivatisation and decarboxylation) targeted different matrices. The HSGC technique appeared to determine total TCAA present whereas the extraction and derivatisation technique measured the water- or base-extractable TCAA depending on the procedure. These results suggested that the measured concentrations should not be the same as they relate to different components of the soil.

The reason for these differences is that the extraction step is very inefficient and does not remove all the TCAA from the soil matrix. The results from Section 5.5.4 show that only 5% of TCAA is extracted from soil with water, increasing to 10% for extraction with very strong base. If a similar proportion is assumed to be extractable from all soils (which seems unlikely) then the average TCAA concentration of 9 ng (g fwt)$^{-1}$ determined by HSGC (for all soils except the tree stands at Dunsclair Heights) would be determined as 0.5 ng (g fwt)$^{-1}$ by a derivatisation methodology. This poor extraction efficiency may explain the reason no soil of greater than 1 ng g$^{-1}$ were reported during a European study using a derivatisation technique (Franklin, J.: Pers. Comm.). A Canadian study (Scott et al. 2000), using a derivatisation technique (Scott & Alaee 1998), determined TCAA concentrations in soils from Britain, Europe and world-wide that were greater than 1 ng g$^{-1}$. It is apparent that soil results
determined using different extraction steps within the derivatisation techniques are variable and so results should be cross-checked to ensure that the derivatisation methods determine the same concentrations at different laboratories. Recent work (Urbansky 2000) suggested that acidified-methanol derivatisation outperforms the diazomethane method as the latter suffers from photo-promoted side reactions. These side reactions should be investigated as they may affect the accuracy of the diazomethane derivatisation method. The opportunity exists for a comparison between HSGC and derivatisation methods to determine the bound and the extractable TCAA in soil. An investigation of the differences in extraction efficiency with pH might also provide information about the binding of TCAA to the soil. A co-operative approach might yield the biggest return of information and help the study of TCAA in the environment. It is not until all the methods are inter-compared, and any differences explained, that a meaningful discussion of results at different locations can be had.

The origin of the CHCl₃ produced from decarboxylation of soils is also of great debate. Complete decarboxylation of TCAA has been proved using standards, but Schöler (Pers. comm.) has suggested that the origin of some CHCl₃ from soil is by microbial action, activated if the temperature exceeded 90°C. This was tested in Section 5.5.4.3 by using decarboxylation conditions of 65°C for 72 hours alongside the normal decarboxylation conditions of 100°C for 90 minutes, on parallel samples of the same soil. Since the same concentrations of CHCl₃ are determined by either procedure, hence disproving Schöler’s hypothesis is disproved. As discussed above, it is likely that the majority of the difference in the measured concentrations between the techniques is explained by the inefficiency of the extraction step. Therefore, if water extractable TCAA concentrations were determined by HSGC similar results would be expected by derivatisation. The only absolute way to test the accuracy of the HSGC method for the analysis of TCAA is to analyse extracts of the same soils using a derivatisation methodology and HSGC. This would establish if the extractable TCAA in the soil was the same by both methods and if it was successful a procedure to compare the total TCAA could be designed. Comparisons between techniques have been published in the literature (Frank et al. 1990) and suggest that
results for wet input and needles by the different techniques give good agreement. The results determined during this research are in the same range as those reported for needles and rain in the literature by either method.

Analysis by HSGC rather than by derivatisation does have its limitations. The main drawback of a HSGC approach is that only TCAA can be determined and other haloacetic acids that may be present cannot be quantified. An attempt was made to decarboxylate dichloroacetic acid (DCAA) to dichloromethane (CH$_2$Cl$_2$) but this was unsuccessful, so it seems that efficient decarboxylation is limited to TCAA. It should be noted that an ECD is less sensitive to CH$_2$Cl$_2$ than to CHCl$_3$, since it contains fewer chlorine atoms, so even if decarboxylation of DCAA were possible the detection limit would be poorer.

The development of a simple method to determine the TCAA present in air samples has been a major achievement. The preparation, operation and analysis of denuders is considerably more involved than the use of glass fibre filters. It is still unclear if the differentiation of particulate and gas-phase TCAA is possible and this would be an interesting area for further work. The long term sampling programme presented here is the first in the literature, and the concentrations determined in Great Britain (max: 100 pg m$^{-3}$) are lower than those measured in Germany (30 – 300 pg m$^{-3}$). The trend of higher TCAA in air in the city of Edinburgh compared to a rural site in Scotland was the opposite of that found in Germany (Hoekstra et al. 1999a). The reasons for this are unclear, but increased local sources of chlorinated solvent precursors or TCAA from combustion processes should be investigated. Reports of high TCAA concentrations in flue gases (Mowrer & Nordin 1987) would suggest that both gaseous and particulate material from combustion processes are potentially significant sources of TCAA.

### 9.2 TCAA in needles of Sitka spruce

TCAA has been monitored at monthly intervals at two sites in Glentress Forest, in at least 4 year classes of needles. A distinctive pattern of increased TCAA concentration with needle age at Dunslair Heights is in contrast to the consistent
concentrations measured in the C to C+3 year needles at Venlaw. These patterns have recurred throughout the growing season and may be indicative of biological degradation processes. This has been explained at Dunslair Heights by possibly greater metabolism in younger needles, and by greater uncontrolled water loss in older needles leading to greater transpiration and uptake of TCAA. Otherwise translocation of TCAA within trees to older needles may be responsible. However, these same explanations do not hold for the trees at Venlaw, where the opposite pattern is observed. Differences in the needle mass loss on drying characteristics (Cape & Percy 1996) at different ages might demonstrate whether the uncontrolled water loss of old needles at Dunslair Heights is different from those at Venlaw. Since an effect could possibly be caused by increased input of pollutants at the more elevated site. The effect of cloud at the top site, which is absent at the lower site, may also lead to different pollutant exposure pathways between the two sites.

Differences in the rates of TCAA metabolism in needles can only be demonstrated using isotopically labelled TCAA. Monitoring the metabolites of TCAA with or without the isotopic labelling could help to determine the metabolic characteristics in needles of different ages or at different temperatures/seasons. The metabolites of TCAA in trees are still not known, as some suggest breakdown to CO₂ and chloride (Matucha: Pers. comm.) whereas others suggest CHCl₃ is formed (Frank et al. 1991; Plümacher 1995). Controlled seedling exposure experiments that intake measurement of all outputs, followed by mass balance calculations, would further the understanding of internal tree processes. Studies using radio-labelled TCAA have used the detection of radioactivity, which only indicates the location of the radio-label and not its chemical form in that location. Uhlírová et al. (1996) and Matucha et al. (2000a; 2000b) have proved that the radio-label is located in the needles following uptake from the soil and distribution of TCAA to needles in a few hours. In future it is necessary to determine the identity and chemical form of this ^14C label.

The discovery of the variation in TCAA concentrations in needles over the growing season at Glentress Forest has also suggested that TCAA is metabolised within trees. If the metabolites of TCAA could be identified with certainty then monitoring them
over time would provide information on the seasonal processing of TCAA. Experiments to attempt to overload this metabolic process would be useful to establish safe environmental concentrations or no observable effect levels, which could in turn be related to any observations of physiological damage such as needle loss or tree death. No objective relationship between needle TCAA concentrations and tree damage has been demonstrated and if this were possible the true environmental significance of TCAA could be established.

9.3 TCAA in soil

The discovery of high TCAA concentrations in forest soils has been the most significant finding of this research and has caused the greatest scientific debate. The idea of natural formation of TCAA in the soil was proposed several years ago (Haiber et al. 1996; Hoekstra et al. 1999b), but the limited measurements in the literature indicates that few people have investigated its validity experimentally. The optimum pH for formation by chloroperoxidase enzymes have been determined as pH 3 to 3.5 (Asplund et al. 1991), which is lower than typically found in Glentress Forest soils.

The high soil TCAA concentrations reported in this thesis have always occurred beneath tree stands in organic rich soils. However, not every soil sampled in a forest stand produces such large concentrations. The recent study in Europe (Franklin, J.: Pers. comm.) has suggested that 1 ng g\(^{-1}\) was the maximum limit of TCAA detected in soil, but this value is exceeded in every sample in this study. The large variability in TCAA concentrations determined over very small distances illustrate the heterogeneity of the soil under tree stands. In striking contrast, however minimal variation was found in the non-tree stand samples at Dunsclair Heights over the whole 12 month period, with mean TCAA concentrations of 7.9 ± 2.7 ng (g fwt\(^{-1}\)) TCAA (n=12). This consistent concentration of TCAA was also determined in moorland soil situated within 2-5 metres of the tree stands that exhibited soil concentrations in excess of 200 ng (g fwt\(^{-1}\)) TCAA. Similarly, at Venlaw, where the total organic matter content of the soil (11 ± 2 % fwt) is typically lower than at Dunsclair Heights (18 ± 3 % fwt), the soil TCAA concentration is constant at 10.0 ± 7.0 ng (g fwt\(^{-1}\))
over 1 year (n=14). This suggests that it is not just the presence of trees that increases the soil concentrations, but some other property of the soil at Dunslair Heights, possibly some functional groups of the organic rich soil. Measurements have shown that soil pH is not significantly lower under the trees than in the open moor or on the rides so it appears unlikely to be a factor in determining soil TCAA.

The high soil concentrations of TCAA that are present have been shown to be non-extractable from the soil matrix using water. This poses the question of whether these soils are very efficient at capturing TCAA from wet input or whether they have higher intrinsic formation rates. The total organic matter (TOM) of the soils under the tree stands is quite constant and so the cause of high TCAA concentrations is more likely to be differences in the functional groups or chloroperoxidase activity in the soil. When the forest site was first established, trees were planted into ploughed furrows. This may explain the high degree of heterogeneity at this site. Dunslair Heights also possesses a thick needle litter layer beneath the stands, which may influence TCAA formation processes. It would be useful to comprehensively characterise one stand with intensive sampling to discover the variability of the TCAA concentrations. The effect of the thick needle litter layer on TCAA concentrations should also be investigated.

Differences in their humic and fulvic acid content or functional group make-up of soils with different TCAA concentrations might highlight the differences in soil structure responsible for TCAA formation/accumulation. Similarly sequential extraction may highlight the matrix in the soil to which TCAA is bound. The application of isotopically labelled TCAA to soils, either in the environment or in the laboratory based studies, would help to characterise binding and metabolism processes. If a source of labelled chloride was introduced to the soil then the incorporation of it into TCAA might also elucidate formation processes. To date the role of soil in TCAA cycling is far from well understood, but the work in this thesis has highlighted many avenues for further research.
9.4 TCAA in wet and dry deposition

Temporal patterns of TCAA concentrations in, and the input to forest by cloud, rain and air have been presented for Glentress Forest. There is no evidence of a seasonal pattern in the concentration of TCAA in rain and cloud samples in contrast to what has been reported in some Central European studies. A more meaningful expression of concentration, the volume-weighted concentration, has been presented which is not biased by small rain or cloud events that may possibly have anomalously high TCAA concentrations. The inclusion of abnormally high concentrations found in small volume samples in previous studies could explain the seasonal patterns found in Europe, where summers are often hot and dry, and experience a marked seasonal variation in wet deposition. The site in Scotland has a more constant distribution of wet deposition throughout the year with peak rainfall found in August and October 1998 and December 1999 during the sampling period (Figure 6.4). It should be noted that the total wet deposition from May to September 1999 is very similar to the period November 1999 to February 2000, which suggests why no effects of season on TCAA concentrations are seen.

Cloud has been sampled and analysed for TCAA throughout the research period. The volume-weighted concentrations are highest in cloudwater during March and April 1999 and March 2000. It is not known why these spring dates should have produced the highest concentrations but there appears to be an inverse relationship between the concentration of TCAA in cloudwater and the volume sampled. This could be simply a consequence of sampling at a fixed height within clouds with varying cloud base and liquid water content.

Some sampling dates have shown enhanced concentrations of TCAA in cloud with respect to rain, but not consistently so. A pattern showing general enhancement in cloud concentrations was expected, as this is found for other anionic species at Glentress Forest. The fact that this was not generally the case for TCAA meant that the TCAA input from cloud was less significant that the input from rain. The latter accounted for 89% of the wet input of TCAA at Dunslair Heights over the 12 month
The different behaviour of TCAA in clouds from that of the other major ions is interesting and should be investigated.

The availability of archived cloud and rain samples dating from the late 1980s to the present day means that an investigation of trends in TCAA over time is a very worthwhile future project. The reduced emissions of the chlorinated precursors of TCAA have led to the expectation of reduced TCAA concentrations in wet deposition with time. If a time-trend could be produced then the general direction of the trend of TCAA concentrations could be distinguished from the observed week-to-week variation. The goal would be to correlate TCAA concentrations in wet input with atmospheric 1,1,1-trichloroethane and/or tetrachloroethene concentrations. Such a study would have to assume negligible degradation of TCAA in stored rain samples, but could provide a lower boundary to the rate of decrease of TCAA in rain over time for comparison with the known large decreases in precursor concentrations.

Another interesting observation from Scott et al. (1999), and supported by Berg et al. (2000) has suggested that sequential sampling during rain events results in the detection of reduced TCAA concentrations as the event continues over time. This phenomenon could be investigated automatically or manually during rain events. This would have significant implications for further work as the time since an air mass last precipitated would be a factor in the deposition of TCAA to a site. The apparent finding that concentrations of TCAA in rain in Scotland are higher than those in Central Europe suggests that the predominantly westerly rain-bearing air masses contain the highest concentration of TCAA, which become gradually depleted as they progress eastwards. An interesting programme would be to study an individual air mass as it passes across Europe, monitoring the rain TCAA concentration as it progresses. Another experiment would be to measure the concentrations of TCAA in individual rain events and relate these concentrations to the direction from which the air masses approaches. This might indicate differences in TCAA concentrations in air masses that originate from Europe or from the
Atlantic, which may be caused by differences in water content or the input of pollution from cities in Europe.

The pattern of greater concentrations of TCAA in urban air compared with rural air is the reverse of that observed in Europe. Samples taken concurrently in Edinburgh gave higher values for gas-phase only results than the total of gas-phase plus particulate measured at Dunslair Heights. The comparison between urban and rural concentrations should be monitored at different sites, this time using the same sampling protocol and site elevation. Two possible reasons for this pattern are elevated local concentrations of chlorinated solvent precursors or as yet unidentified urban sources of TCAA in the gas-phase or particulate matter. The presence of point sources of chlorinated solvents such as industry, dry cleaning and chemical research may increase the local chlorinated solvent air concentrations, but it was thought that the production of TCAA from its solvent precursors would be slow and any local emissions of solvents would be quickly averaged out in the boundary layer and transported by air masses. If this is not the case vastly different concentrations of TCAA would be expected in the precipitation and air of urban environments relative to rural ones. Supporting evidence for this was the huge concentration of TCAA found in rain at Siegen in Germany (Haiber et al. 1996), which was blamed on local industry. However, the higher air concentrations reported in forested sites (Hoekstra et al. 1999a) are in contrast to the results from Dunslair Heights.

The second possible explanation for the differences between rural and urban air could be the increased particulate in cities. The particulate may be demonstrated as a source of TCAA but may also act as a site for adsorption after formation of TCAA. If the airborne particulate in cities has its origin from power production, incineration and combustion engines then, as suggested by Hoekstra et al. (1999a), TCAA might be formed according to the trace chemistries of fire theory (Bumb et al. 1980). Could this be the origin of TCAA in urban environments? If TCAA were able to adsorb to particulate matter then the higher amount of particulate present in Edinburgh would have associated TCAA, which may therefore lead to higher total TCAA in air concentrations in the city. A final suggestion about the higher TCAA
concentrations in urban air measurements is that the rainfall measured in Edinburgh in the year until April 2000 was 657 mm (UK Meteorological Office), whereas at Dunslair Heights it was 1062 mm. This drier climate in Edinburgh may explain some extra contribution of TCAA in the gas-phase, rather than being dissolved and rained out of the atmosphere.

The distinction made at the Edinburgh site between gas-phase and particle-bound TCAA was designed to estimate the contribution of each to the overall measured concentration. The sampling protocol was not designed to capture particulate efficiently, so the particulate contribution is probably underestimated in Edinburgh (Chapter 6). It has been suggested (Barrie, L.: Pers. comm.) that if TCAA were present on particles, after particulate capture TCAA might be volatilised and subsequently re-captured on the back filter and erroneously determined as the gas-phase fraction. A discussion about this led to the conclusion that, as TCAA is often used as a precipitating agent in protein technology, it is likely to be sufficiently sticky to remain adsorbed to particles. A study of the contribution of each fraction of particulate matter to the total TCAA would be interesting as this would allow a comparison of the concentrations measured by this new filter technique and by the established denuder methodology. A monitoring programme of TCAA concentrations in air near a paper mill would also be of great interest as it has been suggested that high local concentrations of chlorine species (from paper bleaching chemicals) might increase TCAA formation (Juuti et al. 1995). No conclusive evidence of this has yet been demonstrated by monitoring needle concentrations, so a survey of air concentrations might be expected to give more direct data than the use of needles as passive samplers, which may degrade some of the captured TCAA.

9.5 Tree growing experiments

The tree growing experiments will be continued, to yield as much information about the effects and pathways of TCAA uptake and to look for long-term damage to the trees that have already been treated. The main finding from the experiment so far is that there are clearly routes of TCAA uptake from both an above-ground and a soil pathway. This has been reported in previous experiments (Sutinen et al. 1995;
1997), but the actual mechanism for uptake has never been investigated. The experiment presented in this thesis has suggested that the above-ground pathway is as efficient at increasing needle TCAA concentrations as the soil pathway and that the above-ground route may be via stem or branch rather than needles. The evidence of higher TCAA concentrations in the stems of foliage treated trees suggests that transfer through, or fixation in, bark is possible. No evidence for transport across stomata has been observed. The use of isotopically labelled TCAA for treatments could help determine the origin of the TCAA detected in the needles.

An improvement to the protocol used in the needle experiments would be to use rain or stream water to irrigate the trees in order to further reduce the concentrations of TCAA in the control treatments and to make changes in needle concentrations arising from exposure at the lower concentration (10 ppb) easier to discern. Continued treatment at environmental concentrations would be further enhanced if a more time-resolved analysis was performed. Fast changes in needle concentrations after the exposure to TCAA might be detected and the uptake kinetics, including metabolic effects, by both pathways elucidated. Some attempts might be made to overload the metabolic mechanisms to determine the lethal concentrations to the tree, or to study the measurement of the metabolites formed after degradation using isotopically-labelled compounds. The question of whether the application of labelled TCAA to seedlings leads to labelled CO₂ or CHCl₃ being released can be investigated. An alternative one dose acute treatment could be used rather than chronic exposure to low TCAA concentrations, and the change in needle TCAA concentrations with time monitored.

The effects of TCAA treatment on tree health have been monitored subjectively during this experiment. No tree deaths in this seedling experiment have been attributed to TCAA treatment even at the 100 ppb level. Matucha has presented work from seedling experiments (Haloacetic Acids Conference, Toronto, 2000), which showed that seedlings experienced harmful effects and died during the growing season after treatment with TCAA after a single high exposure. This has not been the case for the experiment in Edinburgh. The have trees thrived and
produced healthy new needles in the year 2000 growing season. The tree health will continue to be monitored when exposure to TCAA continues.

The health of the seedlings was also examined at a more microscopic level. Biochemical indicators of damage, such as the protein content of needles and roots and needle glutathione-S-transferase activity, have been measured. Significant reductions in the protein content of needles and roots have been correlated to the level of exposure to TCAA, which has been related to the herbicidal properties of TCAA. The activity of the detoxification enzyme GST was significantly increased after the foliar treatment of TCAA. This may be related to a sensitive response of GST to TCAA, even in the control treatments. Toxicological effects of TCAA are therefore useful to elucidate the mechanism that trees use to protect themselves from xenobiotics and how they metabolise these harmful compounds. Though this area of toxicology has been studied (Ashton & Crafts 1973) there is still no clear understanding of the mode of action of TCAA at environmental concentrations. The fact remains that the concentrations in tap water are far higher than those in rain and cloud, yet trees in gardens watered by domestic supply are not dying or showing the effects of forest decline. The role of soil in the interception and immobilisation of TCAA, reducing its availability, may explain the phenomenon, but if TCAA is to be considered a serious environmental pollutant then the toxicology and uptake mechanisms must be more firmly established.

9.6 Co-operative further work

The interest in haloacetic acids (HAA), particularly TCAA, as environmental pollutants is world-wide and results have been presented from Chile, Malawi, Antarctica, the Middle East, North America and Europe. The problem of HAAs is a global one and the release of chlorinated precursors in one part of the world may influence the environment far away. The role of TCAA as an intermediate after the release of volatile and semi-volatile compounds is a stark reminder of the fate of compounds in the environment. However, proof that atmospheric TCAA, possibly originated from the chlorinated solvent industry, is responsible for the concentrations found in other environmental compartments is still missing. The role of soil in
A co-operative world-wide comparison of analytical methodologies is essential to prove that the results that are produced are meaningful and worthy of discussion. The strengths of different analytical methods should be utilised, so as to maximise the understanding of the topic. If other atmospheric intermediates such as trichloroacetaldehyde are present in the environment are they related to industrial chlorinated solvent precursors, and is their lifetime long enough to eventually lead to the production of TCAA?

A European study to monitor the deposition and HAA content of an air mass as it crosses Europe would be an interesting project. A similar Europe-wide experiment has been performed with soils, where a transect was analysed across Europe, but there have been no similar studies in the atmosphere. Even retrospective comparisons of rain from air masses that have passed over different countries would be possible using back trajectories.

A huge variability in TCAA concentration has been encountered in soils at a single site, so it is unlikely that a random collection of soil samples in a few countries will adequately characterise every soil in Europe. It is possible that certain conditions must exist for the natural formation of TCAA and these may occur only in certain areas. Ideally, some of the high TCAA samples from Dunslair Heights should be analysed by other laboratories in Europe. A future goal would be for a proper comparison at Edinburgh of TCAA concentrations in the same soils using the different analytical methods. This would lead the way for appropriate development and research work to explain any differences.

A more complete mass balance study similar to that outlined in Chapter 8 will be performed using the knowledge gained during this research at a different site in Scotland. A more complete mass balance could be delivered if a site, where the
geology and hydrology had already been well characterised, was used. The Ballochbeatties burn in Ayrshire, South-West Scotland is a forested catchment on the banks of the Loch Bradan reservoir, which will be used for a mass balance of TCAA in a forest ecosystem. The aim of the project is to perform a planned sampling and analysis programme to measure the concentration and fluxes of TCAA in the catchment. Sampling of compartments such as throughfall, stream water and soil pore water, that has not been possible in the research project at Dunsclair Heights, and will allow a fuller and more accurate picture of TCAA cycling in the catchment to be established by the use of lysimeters and flow proportional stream samplers, which were not available for the Glentress Forest sampling programme. Additional measurements such as the variation in the concentration of chlorinated precursor compounds will be performed for an investigation of the relationship between TCAA and its possible atmospheric sources. This further research will permit a more coherent discussion of the TCAA cycling processes occurring in the environment, which in the literature to date has not been based on as complete, scientific methodology. This type of study is of interest to both the scientific community, industry and European Environment Agencies and will build on the preliminary findings from the research presented in this thesis. The well planned nature of this programme, which has already been funded by NERC, will facilitate a smooth transition between an exploratory study of a particular site and a comprehensive survey of a well-defined catchment.
References


Appendix A

A worked Analysis of Variance with Tukey's Studentised Range Test

The following example illustrates the statistical method employed for analysis of variance (ANOVA) using the SAS statistical software. The worked example shows how the ANOVA is split into its degrees of freedom including all the interaction terms and run. If there is no significance to the interaction terms they are removed sequentially in order of their contribution to the sum of squares. If there is no significance to the interaction terms they are removed and the model finally run with only the combination of the main effects which are significant in the initial ANOVA. The remaining degrees of freedom are used as error terms in evaluating significant difference. The worked example is based on the data for Glutathione protein measurements in seedling needles shown in Figure 7.8. Initially the data are split into their contributions to the degrees of freedom (Table A1).

<table>
<thead>
<tr>
<th>Class</th>
<th>Symbol</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level</td>
<td>l</td>
<td>3</td>
<td>0, 1, 2</td>
</tr>
<tr>
<td>Treatment</td>
<td>t</td>
<td>2</td>
<td>1, 2</td>
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<tr>
<td>Batch</td>
<td>b</td>
<td>4</td>
<td>1, 2, 3, 4</td>
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</table>

Table A1; Class level information

The ANOVA is calculated using the protein content as the dependent variable as shown in Table A2. There are 48 observations in the model.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>ANOVA SS</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr &gt; F</th>
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<tbody>
<tr>
<td>b</td>
<td>3</td>
<td>0.07907081</td>
<td>0.02635694</td>
<td>3.67</td>
<td>0.0231</td>
</tr>
<tr>
<td>l</td>
<td>2</td>
<td>0.06449293</td>
<td>0.03224647</td>
<td>4.49</td>
<td>0.0198</td>
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<tr>
<td>t</td>
<td>1</td>
<td>0.01744600</td>
<td>0.01744600</td>
<td>2.43</td>
<td>0.1298</td>
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<tr>
<td>l*b</td>
<td>6</td>
<td>0.03927415</td>
<td>0.00654569</td>
<td>0.91</td>
<td>0.5008</td>
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<td>t*b</td>
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<td>0.00276409</td>
<td>0.00092136</td>
<td>0.13</td>
<td>0.9427</td>
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<tr>
<td>l*t</td>
<td>2</td>
<td>0.00641858</td>
<td>0.00320929</td>
<td>0.45</td>
<td>0.6441</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>0.21569355</td>
<td>0.00718979</td>
<td></td>
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Table A2; Two-way ANOVA results for protein content in seedling needles (1)
Tukey’s Studentised Range Test is now performed for protein content using the parameters in Table A3.

<table>
<thead>
<tr>
<th>Parameter</th>
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<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
<tr>
<td>Error Degrees of freedom</td>
<td>30</td>
</tr>
<tr>
<td>Error mean square</td>
<td>0.00719</td>
</tr>
<tr>
<td>Critical value of studentised range</td>
<td>3.84540</td>
</tr>
<tr>
<td>Minimum significant difference</td>
<td>0.0941</td>
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Table A3; Tukey’s Studentised Range Test parameters

The results are shown in Table A4, A5 & A6 where means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Tukey grouping</th>
<th>Mean Protein (mg ml⁻¹)</th>
<th>n</th>
<th>Batch</th>
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<tbody>
<tr>
<td>A</td>
<td>0.27759</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>A/B</td>
<td>0.24114</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>A/B</td>
<td>0.22898</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>0.16510</td>
<td>12</td>
<td>4</td>
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Table A4; Tukey grouping by Batch for needle protein content

<table>
<thead>
<tr>
<th>Tukey grouping</th>
<th>Mean Protein (mg ml⁻¹)</th>
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<tr>
<td>A</td>
<td>0.27833</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0.21459</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>0.19169</td>
<td>16</td>
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Table A5; Tukey grouping by Level for needle protein content

<table>
<thead>
<tr>
<th>Tukey grouping</th>
<th>Mean Protein (mg ml⁻¹)</th>
<th>n</th>
<th>Treatment</th>
</tr>
</thead>
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<tr>
<td>A</td>
<td>0.24727</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>0.20914</td>
<td>24</td>
<td>1</td>
</tr>
</tbody>
</table>

Table A6; Tukey grouping by Treatment for needle protein content
The ANOVA (Table A2) clearly shows that the interaction terms are not significant and the effect of treatment is weakly significant. Therefore the interaction terms are removed and the model is re-run. The new ANOVA results are shown in Table A7.

<table>
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<tr>
<td>b</td>
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<td>0.02635694</td>
<td>4.09</td>
<td>0.0125</td>
</tr>
<tr>
<td>l</td>
<td>2</td>
<td>0.06449293</td>
<td>0.03224647</td>
<td>5.01</td>
<td>0.0114</td>
</tr>
<tr>
<td>t</td>
<td>1</td>
<td>0.01744600</td>
<td>0.01744600</td>
<td>2.71</td>
<td>0.1075</td>
</tr>
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<td>Error</td>
<td>41</td>
<td>0.26415037</td>
<td>0.00644269</td>
<td></td>
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</table>

Table A7; Two-way ANOVA results for protein content in seedling needles (2)

The new ANOVA results show that the batch and level terms are more significant than previously, but the treatment term is still only weakly significant (P > 0.1). The treatment term is now removed, the interaction term with the most significance from Table A2 (l*b) included and the model re-run. The new ANOVA results are shown in Table A8.

<table>
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<th>Source</th>
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<td>0.03927415</td>
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<td>0.4578</td>
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<td>Error</td>
<td>36</td>
<td>0.24232222</td>
<td>0.00673117</td>
<td></td>
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</table>

Table A8; Two-way ANOVA results for protein content in seedling needles (3)

The effect of the interaction term is to slightly reduce the significance of the batch and level terms. The level*batch interaction term still remains very insignificant (P>0.45). The interaction term is removed and the model is finally run with only the significant batch and level terms. The final ANOVA is shown in Table A9. The final results from Tukey’s Studentised Range Test are shown in Table 7.11, 7.12 & 7.13. There is clearly a significant effect (P<0.02) of Batch and Level on the protein content in the needles of the seedlings.
<table>
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<th>F value</th>
<th>Pr &gt; F</th>
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<tr>
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<tr>
<td>l</td>
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<td>Error</td>
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</tbody>
</table>

Table A9; Two-way ANOVA results for protein content in seedling needles (4)
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Figure 4.4 Temporal pattern in needle TCAA concentrations at Dunslair Heights during 1998 growing season

Figure 4.5 Temporal pattern in needle TCAA concentrations at Venlaw during 1998 growing season

Figure 4.6 Temporal pattern in needle TCAA concentrations at Dunslair Heights during 1999 growing season

Figure 4.7 Temporal pattern in needle TCAA concentrations at Venlaw during 1999 growing season

Figure 4.8 Temporal pattern of TCAA concentrations in current year (1998) needles at Dunslair Heights

Figure 4.9 Temporal pattern of TCAA concentrations in current year (1998) needles at Venlaw

Figure 4.10 Temporal pattern of TCAA concentrations in C+2 year (1996) needles at Dunslair Heights

Figure 5.1 The formation of CHCl₃ and TCAA from a resorcinolic structure

Figure 5.2 A map showing sampling locations at Dunslair Heights

Figure 5.3 TCAA temporal variation in soil from open moor at Dunslair Heights (1999-2000)

Figure 5.4 TCAA temporal variation in soil from rides at Dunslair Heights (1999–2000)

Figure 5.5 TCAA temporal variation in soil from Venlaw forest (1999-2000)

Figure 5.6 TCAA temporal variation in soil from tree stands at Dunslair Heights (1999-2000)

Figure 5.7 TCAA temporal variation in soil from tree stands at Dunslair Heights (1999-2000) [0-250 ng/g scale]

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Figure 5.11 TCAA soil extraction experiments (16 hours)
Figure 5.12 Soil extraction experiments with acidic, neutral and basic conditions
Figure 5.13 Soil TCAA degradation experiment at room temperature and 4°C
Figure 5.14 Relationship between TCAA and total organic content in soil at all sites in Glentress Forest
Figure 5.15 Relationship between TCAA and water content in all soils at Glentress Forest

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Figure 6.2 Air sampling apparatus at Dunslair Heights
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Figure 6.4 Monthly wet deposition pattern in Dunslair Heights rain
Figure 6.5 Monthly wet deposition pattern in Dunslair Heights cloud
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Figure 6.7 Rainfall weighted monthly average TCAA concentration in Dunslair Heights rain (June 1998 to April 2000)
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