Uptake of Airborne Organic Pollutants in Pine Needles

Geographical and Seasonal Variations

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2003

Acta Universitatis Agriculturae Sueciae
Agraria 402

ISSN 1401-6249
ISBN 91-576-6415-3

Tryck: Kista Snabbtryck, Kista 2003

Abstract

Hellström, A. 2003. *Uptake of airborne organic pollutants in pine needles – geographical and seasonal variations.* Doctor's dissertation.
ISSN 1401-6249, ISBN 91-576-6415-3.

The fate and distribution of semivolatile organic pollutants (SOC) and has received much attention during the last decade. The aim of this thesis is to address key issues concerning the fate of some selected pollutants in the terrestrial environment. Specifically, several aspects of the uptake of airborne organic pollutants in pines has been studied.

The geographical distribution of some organochlorines in pines was investigated in a transect from Central to Northernmost Europe and also in the former Eastern Europe. Most compounds with a recent active use in Europe showed higher concentrations in the south than in the north. However, the industrial by-product hexachlorobenzene had elevated levels close to industrial centra, and α-hexachlorocyclohexane (α-HCH) had similar concentrations throughout. To properly interpret such data the variability within a forest stand was investigated for selected compounds. The relative standard deviation was found to be better than 25 % for all analytes. The accumulation during the life span of the needles and the seasonal uptake pattern was investigated. A significant uptake during the warm season was found for the most volatile of the analytes, while little happened during winter. This is contrary to what would be expected from physicochemical considerations. It was therefore concluded that there must be a biological factor that alters the properties of the needles so that the accumulation is highest in summer. The least volatile analytes had a transient concentration peak during winter, and analytes with intermediate volatility showed an intermediate behaviour. To test if the terpene concentration in the needles could be the biological factor that explains the uptake pattern of the most volatile compounds, the mono- and sesquiterpene concentrations were measured. It was found that although the total terpene concentration in the needles did not covary with the uptake of α- and γ-HCH, the concentration of terpenes in the soluble cuticular lipids showed peak concentrations during summer, at the same time as the highest uptake of the volatile analytes is expected.

Key words: Pinus sylvestris, hexachlorocyclohexane, POP, DDT, PCB, HCB, mono- and sesquiterpene, biomonitoring, bioaccumulation.

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Appendix

Papers I-IV

This thesis is based on the following papers, wich will be referred to in the text by the respective Roman numerals.

I. Kylin, H., Hellström, A., Nordstrand, E. & A. Zaid, 2003. Organochlorine pollutants in scots pine needles — biological and site related variation within a forest stand. *Chemosphere* 51, 669-675.

II. Hellström, A., Kylin, H., Strachan, W.M.J. & Jensen, S. 2003. Distribution of some organochlorine compounds in pine needles from Central and Northern Europe. (submitted).

III. Hellström, A., Kylin, H. & Sjödin, A. 2003. Seasonal variations in the uptake of airborne gaseous and particle bound semivolatile organic compounds in Scots pine needles. (Manuscript).

IV. Hellström, A. & Kylin, H. 2003. Terpenes, an overlooked factor in uptake of airborne pollutants in Scots pine needles? (manuscript).

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Introduction

Man made organic pollutant are spread all over the world although they have been produced, used and dispersed for a quite short period of mans history. Today pesticides and industrial chemicals are found even in such remote areas as Arctic and Antarctic (France *et al.*, 1997; de March *et al.*, 1998) .

Plants make up a large hydrophobic surface in contact with air and may act as a sink for airborne organic pollutants and affect long range transport of hydrophobic pollutants, particularly in terrestrial ecosystems. It is, therefore, possible that plants play an important role in the fate of organic pollutants in the terrestrial environment. Conifers are well suited for studies of pollutant levels because of their evergreen character and often long-lived foliage. Sometimes the needles have a life cycle of several years. Conifers are widespread and common in the Northern Temperate Zone, and are dominant species in the boreal forests. Therefore conifers have been widely used for studies on accumulation of airborne pollutants.

Scots pine (*Pinus sylvestris*) is a common species in northern Europe. It is also fairly simple to distinguish between the year-classes of needles in Scots pine. For these reasons, Scots pine has been popular to use in studies of the uptake of airborne organic pollutants in plants.

The accumulation of airborne organic pollutants in Scots pine needles and other conifers have first focused on the use of pines as monitors of airborne organic pollutants (Eriksson *et al.*, 1989; Jensen *et al.*, 1992; Weiss, 1998). In recent years the interest in the role of vegetation in the fate of airborne organic pollutants has growth. The general conclusion drawn from both areas of research is that more knowledge about the mechanism of uptake and accumulation of organic pollutants in plants under field conditions is needed (Kylin, 1994). Therefore, our interest has turned to studying factors governing the uptake and accumulation processes of airborne organic in conifers.

For the papers in this thesis pines needles were used to map the distribution of some airborne organic pollutants in northern and central Europe (Paper II). In this geographical study we analysed all available year-classes of needles to evaluate differences between year-classes of needles. To evaluate the relevance of the sampling procedure the variation among pines from a rather small pine forest was first tested (Paper I). The seasonal variations in accumulation patterns of different pollutants were addressed in a long-term (2.5 year) study of airborne organic pollutants in Scot pine needles in Stockholm (Paper III) and in a study of one airborne pollutant and terpenes, a seasonal varying factor suspected to influence accumulation in pine needles, in parallel (Paper IV).

Aim

To investigate 1) the use of Scots pine (*P. sylvestris*) for environmental monitoring purposes, 2) the role of Scots pine the fate of airborne pollutants, and 3) test the hypothesis that terpenes are one biological factor affecting the seasonal variation of airborne organic pollutants accumulating in Scots pine needles.

Background: Plant uptake of airborne organic pollutants

Airborne organic pollutants

Two properties predisposes a compound to be foundin the environment long after emission and far from the source 1) persistence in the environment, and 2) a sufficient volatility to undergo long-range air transport. To stress those properties two acronyms have been established. Persistent Organic Pollutants are commonly called POPs and pollutants with a semivolatile character (i.e. they can be found in the air both in the gas phase and sorbed to particles) are referred to as SOCs (Semivolatile Organic Compounds). The well known “old” chlorinated pesticides such as DDT and Lindane and compounds emitted from industrial operations, incineration, and other human activities such as the polychlorinated biphenyls (PCB) and polychlorinated dibenzo-p-dioxins (PCDD), or "dioxins" for short, have these properties. The compounds addressed in this work, presented in Figure 1, are α-hexachlorocyclohexane (α-HCH), γ-hexachlorocyclohexane (γ-HCH, also known as lindane), hexachlorobenzene (HCB), 1,1-dichloro-2,2-*bis*(4-chlorophenyl) ethene (DDE), 1,1-dichloro-2,2-*bis*(4-chlorophenyl) ethane (DDD), and 1,1,1-trichloro-2,2- *bis*(4-chlorophenyl) ethane (DDT), and some (six) PCB congeners (CB-28 = 2,4,4'-trichlorbiphenyl; CB-52 = 2,2',5,5'-tetrachlorobiphenyl; CB-138 = 2,2',3,4,4',5'-hexachlorobiphenyl; CB-153 = 2,2',4,4',5,5'-hexachlorbiphenyl; CB-170 = 2,2',3,3',4,4',5-heptachlorobiphenyl; CB-180 = 2,2',3,4,4'5,5'-heptachlorobiphenyl). Throughout this text the term airborne organic pollutants will be used instead.

As for other environmental processes, the hydrophobicity of a compound is very important factor for foliar uptake. Organic compounds with high persistence in the environment are often hydrophobic and are therefore often found in natural lipids such as animal fat and plant waxes. The partition coefficient between octanol and water (K_{OW}) is the common way to describe the hydrophobicity of a compound.

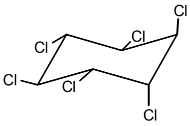
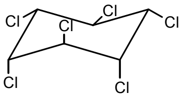
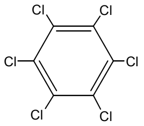
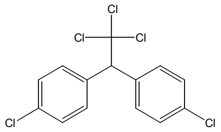
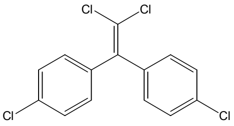
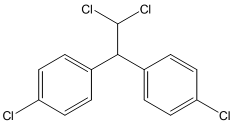
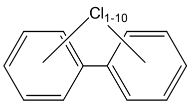
 α-HCH	vapor press (Pa)	0,003
	solubility (g/m^3)	1
	log K_{ow}	3.8
 γ-HCH	vapor press (Pa)	0.00374
	solubility (g/m^3)	7.3
	log K_{ow}	3.6
 HCB	vapor press (Pa)	0.0023
	solubility (g/m^3)	0.005
	log K_{ow}	5.5
 p,p'-DDT	vapor press (Pa)	2×10^{-5}
	solubility (g/m^3)	0.005
	log K_{ow}	6.2
 p,p'-DDE	vapor press (Pa)	8.66×10^{-4}
	solubility (g/m^3)	0.04
	log K_{ow}	5.7
 p,p'-DDD		
 PCB		

Figure 1. Structures of some airborne organic pollutants mentioned in this thesis. Physicalic data from (Beyer *et al.* 2000).

Large hydrophobic compounds are usually not readily taken up from roots or translocated in the plants (Bacci & Gaggi, 1985; 1986; Paterson *et al.*, 1990; Wang & Jones, 1994), with one interesting exception. (Hülster *et al.*, 1994) found transport of dioxin in two species of the genus *Cucurbita*, zucchini and pumpkin. Transport of hydrophobic compounds is limited by their sorption onto plant solids such as the lignificated layer surrounding endodermis of roots. They are also less readily transported by phloem stream because of their low solubility in water. In contrast, the large hydrophobic surface that the plant foliage constitutes an ideal collector for hydrophobic compounds

Octanol-air partition coefficient, K_{OA}, is a parameter used to understand a pollutants sorption to leaf surfaces. K_{OA} can be calculated from Henry's law constant, H′, and K_{OW} or measured (Harner & MacKay, 1995). Keymeulen et. al. (1997) used a headspace method to determinate cuticle-air partition coefficient (K_{CA}) for four monocyclic hydrocarbons and showed good correspondence between log K_{CA} and log K_{OA}. Tolls et. al.(1994) showed a linear correlation between the plant-air partition coefficient and K_{OA}. K_{OA} has been extensively used in modelling of plant uptake from air (Bacci *et al.*, 1990a; Paterson *et al.*, 1991; McLachlan, 1999).

Deposition

The first step in deposition of airborne organic pollutants is transport by wind through free air and by eddy diffusion and sedimentation through the boundary layer above vegetation. Close to the plant surface is a laminar layer were gaseous compounds and small particles (<0.3 µm) are transported only by diffusion, while larger particles undergo sedimentation. The thickness is affected by wind and leaf surface architecture (Bakker, 2002).

Particle bound - gas phase

Airborne organic pollutants can be deposited to plant surface either from the gas phase or bound to particles in air. If and to which proportion a compound is bound to particulate matter in air is dependent on the subcooled vapour pressure of the compound (Bidleman, 1988). Many of the common airborne organic pollutants have vapour pressures in the range (10^{-6}-10^{-2} Pa) that predispose them to be distributed between both the particle and gas phases at ambient temperatures (Eisenreich *et al.*, 1981; Bidleman, 1988). The proportion associated with particles in air will also be dependent on temperature, relative humidity, and the concentration and nature of particulate matter (Bidleman, 1988; Smith & Jones, 2000). Finizion et. al. (1997) showed a correlation between gas particle partitioning of organic compounds and the, compared to the subcooled liquid vapour pressure, more easily accessible K_{OA}. A recent review on transfer of particle bound organic contaminants to vegetation states that there is little information on the wet and dry particulate deposition of organic airborne pollutants to vegetation especially considering field studies (Smith & Jones, 2000).

Particles in air can be divided into three classes (Bidleman, 1988). (1) The one called aitken mode is the smallest (<0.08 µm) and contribute most to the number of particles but little to the total mass owing to their size. Those are formed by condensation of gases and growth by further condensation and coagulation into (2) accumulation mode (0.08-2 µm) particles which constitutes most of the surface area and half of the total mass of typical urban aerosol. Accumulation mode particles are slowly removed from air by rain and dry deposition and have therefore a higher potential for transport over long distances compered to (3) course particles (2-2.5 µm) which are deposited gravitationally fairly rapid.

Course mode particles also have another origin, they are produced by mechanical processes such as blow dust, volcanic material, sea spray and release of pollen and spores and abrasion of the surface wax of plants.

Compounds in both the particle and gas phases can undergo either wet or dry deposition. Wet or dry deposition of particles are often considered together. The dissolution of gaseous compounds into rain or clouds can be estimated with the Henry's law constant (H), an air–water partitioning coefficient determined from the ratio of vapour pressure to water solubility (Boethling & Mackay, 2000).

PCBs seems to be associated with a wider range of particles than the combustion derived polycyclic aromatic hydrocarbons (PAH), in an urban environment PCB showed to be associated with a variety of particles (Holsen *et al.*, 1991; Smith & Jones, 2000). Also PCBs associated with course particles represented a significant fraction of the total PCB deposition. In long-range transport of airborne organic pollutants course particles play a minor role due to their high deposition rate. PCB has also shown to be completely reversible partitioned between particle and gas phase (Simcik *et al.*, 1998).

Welsch-Pausch (1995) and co-workers found that dry gaseous deposition was the principal pathway of tetra- to hexachlorinated polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) in greenhouse and out-door experiments with welsh ray grass (*Lolium multiflorum*). Two field studies made by Simonich et. al. (Simonich & Hites, 1994) and Nakajima et. al. (1995) concerning deposition of PAH to a number of plant surfaces (needles, leaves, seeds, and bark from sugar maple (*Acer saccarum*) and white pine trees (*Pinus strobus*) and azalea leaves (*Rhododendron oomuraski*) led to the same conclusion.

Temperature and wind

Besides particles and precipitation ambient factors that can influence accumulation of airborne organic pollutants into plant is temperature and wind. Temperature was in laboratory experiments shown to influence the partition coefficient for uptake in foliage (Baur *et al.*, 1997; Keymeulen *et al.*, 1997). Partitioning of PCB between air and rye grass at laboratory conditions (using a fugacity meter) showed that under environmental conditions the temperature dependence of the partitioning coefficient did not influence the plant concentration of most semivolatile organic compounds (Kömp & McLachlan, 1997). The slow uptake/clearance kinetics prevent the plant/air system from reacting quickly to the new equilibrium state resulting from the temperature-induced change in the partitioning coefficient, only for relatively volatile SOCs can plant concentration be expected to respond to temperature induced changes in K_{PA} (plant-air partitioning coefficient).

Wind affects the uptake rate by decreasing the thickness of the laminar layer or by increasing eddy diffusion. The air-side resistance has been reported to be the rate-limiting step in uptake of airborne organic pollutants in plants (McLachlan, 1999). However in a recent study also the plant-side resistance was shown to be significant under normal wind conditions (Barber *et al.*, 2002b).

Cuticle

The cuticle is a waxy heterogeneous layer that serves as protection and as a barrier to water loss. The cuticle consists predominantly of soluble and polymeric lipid material, synthesised by epidermal cells and deposited on the outer walls (Figure 2). The cuticular membrane consists of an outer region of soluble and polymerised aliphatic lipids and an inner layer containing large amount of various cell-wall polysaccharides. A pectin-rich layer attaches the cuticular membrane to the cell wall. Principal component of the cuticle membrane is the insoluble lipid component cutin, a polyester of cross-linked hydroxy-fatty acid and hydroxyepoxy-fatty acids only soluble by alkaline hydrolysis of the ester bindings. The major monomer of cutin from Scots pine is 9,16-dihydroxyhexadecanoic acid (Riederer, 1989). Recently the presence of a non-ester lipid polymer in cuticle has been indicted and named cutan (Jeffree, 1996).

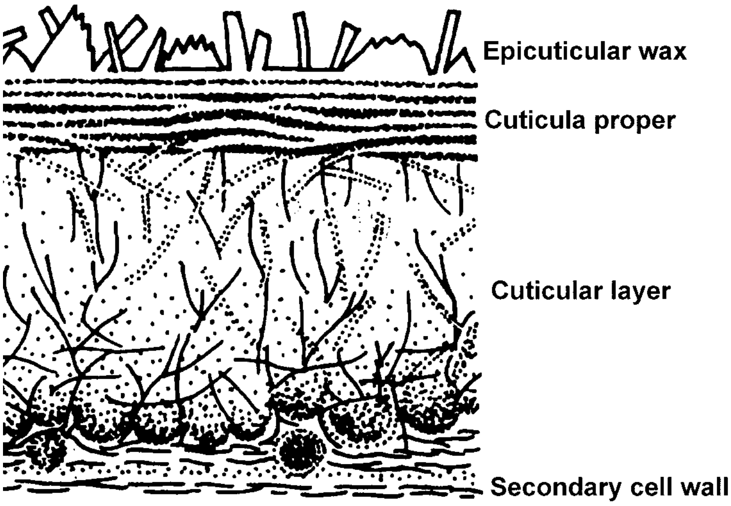


Figure 2. A general illustration of a cuticle to show the heterogen nature of this membrane. (Jeffree 1996, with permission from BIOS Scientific Publishers).

Figure 2 shows the general structure and immense heterogeneity of a plant cuticle. There are substantial differences in chemistry and structure between species. Inter-specific variation in cuticle structures ranging from essentially amorphous to predominantly laminar or reticulate structure (Kirkwood, 1999). However, the knowledge of conifer cuticles seems to be scarce (Jeffree, 1996). Most investigated conifers have no laminar structure. There is no microscopic study of the Scots pine cuticle, only a study of the epidermis ultra structure (Walles *et al.*, 1973). However, the structure of the Norway spruce (*Picea abies*) cuticle is mostly reticulate (Tenberge, 1992).

Wax

Incorporated in the polymeric structure of cutin is the (intracuticular) wax, considered to be the rate-limiting barrier for uptake of airborne organic pollutants into plants. In conifers the wax mainly consists of ω-hydroxy fatty acids (Herbin & Sharma, 1969; Riederer, 1989). Most plant species also have an outer epicuticular wax layer. Chemically different from the inner waxes but difficult to practically separate them by any available methods (Riederer & Markstädter, 1996). In Scot pine needles this has a tubular structure and is believed to be made up of 10-nonacosanol (Riederer, 1989).

The epicuticular wax of pine needles undergoes an ageing procedure during the needle lifetime (Turunen & Huttunen, 1996). The wettability of the surface increase and the tubular structure changes. Pollutants can enhance this ageing process.

Stomata

The actual pathways of airborne organic pollutants into plants are not fully understood. The role of stomata in the uptake process is debated. Theoretical models have shown that the stomatal pathway is negligible compared to cuticular transport for most airborne organic pollutants discussed here because of their high hydrophobicity (Riederer, 1995); (McLachlan *et al.*, 1995). However a recent study shows the possible importance of stomata in uptake of PCBs in *Hemerocallis x hybrida* (Barber *et al.*, 2002a). The stomata of most species in the family Pinaceae (Riederer, 1989) are covered with tufts of tubular wax, which are thought to trap the hydrophobic compounds. Many pine species also have a funnel-like cavity outside of the stomata called the epistomatal chamber or stomatal antechamber filled with dense tufts of tubular wax structures. Therefore the stomatal pathway has been considered less important than cuticular uptake of hydrophobic airborne organic pollutants into pine needles.

Uptake studies

Enzymatically isolated cuticular membranes from leaves and fruits have been used for studies of partitioning, permeability, and diffusion properties (Riederer & Schonherr, 1984; Schönherr & Riederer, 1989; Riederer, 1995; Schreiber *et al.*, 1996). Much of the work is summarised in Schönherr and Riederer (1989), Schreiber et al (1996), Schönherr and Baur (1996), and by Kirkwood (1999). The method most often used is a technique to measure the unilateral sorption from the outer face of an isolated cuticle in a desorption chamber.

A determination of the plant cuticle-air partitioning coefficients with enzymatically isolated cuticles was made by Keymeulen et al. (1997). The method was based on gas phase equilibrium of target compound in closed vials and subsequent analysis by headspace gas chromatography.

According to Schönherr and Riederer (1989), cuticles are heterogeneous membranes and may be looked at as composite membranes made up of a thin outer layer which is the barrier limiting the rate of uptake and transport, and an inner compartment which is responsible for its sorptive properties . The conclusion that the cuticular membrane is not homogenous came from difference between measured and permeability calculated leaf-air partitioning coefficients (Riederer & Schonherr, 1984; 1985). This is also easily shown by permeability experiment of isolated cuticles repeated with only the polymer matrix left after extraction of the soluble cuticular lipids (Schönherr & Riederer, 1989). The permeability increased by a factor ranging from 10 to1000 in intact cuticle membrane compared to in the polymeric matrix. The path length across the heterogeneous cuticular membrane is not simply given by its thickness, but by the tortuosity of the diffusional path within the membrane (Schreiber *et al.*, 1996). The asymmetric nature of the cuticle was also shown by measuring the difference in efflux rate across the inner and outer layer of the isolated cuticle. The efflux rate across the inner surface was 50-80 times larger than across the outer layer (Schönherr & Riederer, 1989). Also the polymeric membrane (MX) obtained after the removal of the soluble lipids proved to be asymmetric in many species.

Terpenes

Terpenes are compounds built up of two or more isoprene (C_5) units commonly found in many plant species. The actual precursor is isopentyl pyrophosphate (Dell & McComb, 1978) which according to recent research can be produced both through a mevalonate or a mevalonat-independent pathway (Rohmer, 1999) in plastides or in the cytoplasma of producing cells.

Many important biomolecules are derived from terpenes, e.g. the carotenoids and steroids. But the smaller mono- and sesquiterpenes are secondary metabolites and was for long considered waste products with no known function. Nowadays their possible functions in host-insect interaction and as defence against insects and fungal attack have been widely studied (Fäldt, 2000). Recent studies have proposed other function such as stablisers in tyhlacoid membranes resulting in higher thermotolerance (Delfine *et al.*, 2000; Singaas, 2000).

The essential oils of conifers consist mainly of volatile mono- and sesquiterpenes. They are secreted into resin ducts after production. The composition of terpenes and especially monoterpenes have been analysed in many conifers (Zafra & Garcia-Peregrin, 1976; Leseche *et al.*, 1982; Rudloff & Granat, 1982; Chalchat *et al.*, 1985; Stepen & Kuznetsova, 1986; Cool *et al.*, 1991; Cool & Zavarin, 1992; Roussis *et al.*, 1995; Rafii *et al.*, 1996; Stepen *et al.*, 1996; Koukos *et al.*, 2000). Most analyses have been done on monoterpenes but there are some reports on the sesquiterpene composition of Scots pine needles (Gornostaeva *et al.*, 1981; Chalchat *et al.*, 1985; Manninen *et al.*, 2002) and other pines (Leseche *et al.*, 1982; Roussis *et al.*, 1995; Koukos *et al.*, 2000) There are also reports on seasonal variation of terpene composition (Rudloff & Granat, 1982; Brooks *et al.*, 1987; Wagner *et al.*, 1990; Stepen *et al.*, 1996; Kylin *et al.*, 2002).

It has been suggested that variations in the amount a possibly the composition of the hydrophobic material in the needles could explain some of the observed seasonal variation in the uptake of the airborne hydrophobic pollutants in pine needles (Kylin *et al.*, 2002; Kylin & Sjödin, 2003). When searching a biological factor that accounted for a seasonal change in the hydrophobic capacity of Scots pine needles mainly mono- and sesquiterpenes were found by gas chromatographic analysis of an extracted material from Scots pine needles called total extractable surface hydrophobic material (Kylin *et al.*, 2002). The total weight percentage of this material constituted up to 12 % of dry weight during summer and only 2 % in winter. There are few other studies of terpenes in the wax layer of needles. Franich et.al. (1993) found mono- and sesquiterpenes at equal amounts in extracts of epicuticular wax of Monterey pine (*P. radiata*). Szafranek (1998) studied sesquiterpene composition in cuticle lipids of potato. There is only one report on terpenoids possible effect on bioconcentration of volatile organic compounds (VOC) (Hiatt, 1998). But the author did not consider the endogenic production of VOCs.

Plant uptake studies

The accumulation of airborne organic pollutants in plants has been an area of interest for a long time. It has been reviewed by Paterson (1990), Kylin (1994), Simonich (1995b), and Smith and Jones (2000). The first attempt to use plants for monitoring airborne pollutants was made by Jensen (1966). PCB in Scots pine needles was investigated in Stockholm and the Ruhr area in Germany. Up to the beginning of the 1990ties, most field studies focused on the use of plants to map the distribution of airborne organic pollutants.

Conifers have been widely used for biomonitoring of airborne organic pollutants (Reischl, 1988; Eriksson *et al.*, 1989; Reischl *et al.*, 1990; Calamari *et al.*, 1991; Jensen *et al.*, 1992; Safe *et al.*, 1992; Strachan *et al.*, 1994; Juuti *et al.*, 1995; Tremolada *et al.*, 1996; Wenzel *et al.*, 1997; Ockenden *et al.*, 1998; Weiss, 1998; Xu *et al.*, 2003) , but also many studies on mosses (Chovanec *et al.*, 1994; Wenzel *et al.*, 1998), lichen (Muir *et al.*, 1993; Ockenden *et al.*, 1998) and other plants (Buckley, 1982; France *et al.*, 1997) have been performed.

Biomonitoring using plants have been performed on local basis to spot emission sources from local industries such as TCA from pulp mills (Juuti *et al.*, 1995), and dioxins from wood-preserving activities (Safe *et al.*, 1992). On regional basis have suspected and unexpected emission sources such as industrial activities and pesticide spraying been detected, geographical trends seen (Eriksson *et al.*, 1989; Jensen *et al.*, 1992; Muir *et al.*, 1993; Strachan *et al.*, 1994; Tremolada *et al.*, 1996; Weiss, 1998), andcomparison between areas made (Wenzel *et al.*, 1997). Global trends in compounds and concentration have also been investigated (Calamari *et al.*, 1991; Simonich & Hites, 1995a). Background levels (Buckley, 1982) and input to remote areas (France *et al.*, 1997) can also be tracked with plants. A comparison of lichen and pines as bioaccumulators of airborne organic pollutants was presented (Ockenden *et al.*, 1998). Most often hydrophobic air contaminants are analysed in leaves or needles, but also the lipids from bark have been used to monitor pollutants (Simonich & Hites, 1995a). An interesting study from China shows that known organochlorines, such as DDT, HCHs, Aldrin, heptachlor and chlordanes, constitute only 1.4-19.8 % of extractable sulphuric acid resistant organochlorine (EPOCl) in pine needles (*P. massonia* and *P.*

tabulaeformis.), indicating a major proportion unknown persistent organochlorine material (Xu *et al.*, 2003).

There are also field studies focusing on uptake mechanism most of them short to medium time studies (McLachlan *et al.*, 1995; Hung *et al.*, 2001; Smith *et al.*, 2001; Barber *et al.*, 2002b) and a few long-term studies, (Simonich & Hites, 1994; Kylin & Sjödin, 2003).

Plant uptake kinetics have been studied in closed laboratory-systems including root uptake (Trapp *et al.*, 1990) or air-plant kinetics only (Bacci & Gaggi, 1985; 1986; Bacci *et al.*, 1990a; Bacci *et al.*, 1990b; Bacci & Calamari, 1991; Bacci *et al.*, 1992; McCrady & Maggard, 1993; Hauk *et al.*, 1994; Tolls & McLachlan, 1994). Data obtained from those systems are used for modelling the whole plant (Hung & Mackay, 1997) or only air-leaf partitioning (Paterson *et al.*, 1991). The kinetic described for the model-system known as the "azalea-model" is a one-compartment model (Bacci & Gaggi, 1985; 1986; Bacci *et al.*, 1990a; Bacci *et al.*, 1990b; Bacci & Calamari, 1991; Bacci *et al.*, 1992). Most of the more recent studies indicate that uptake is best described by a two-compartment model (Reischl *et al.*, 1987; Schreiber & Schönherr, 1992; McCrady & Maggard, 1993; Hauk *et al.*, 1994; Tolls & McLachlan, 1994; Umlauf *et al.*, 1994). Many authors have noted that equilibrium may never be reached during the length of experiment. Paterson (1990) in a review states that steady state is useful to describe the fate of water-soluble chemicals with a log K_{OW} between 1 and 2. However, for more hydrophobic chemicals it is essential to describe these processes by dynamic or non-steady state models since time for uptake may be long comparable to the life of the plant.

The role of plants for the fate of airborne organic pollutants on a local, regional and global scale has received increased attention during recent years. Forests and vegetation in general were considered to have little influence on global transport of airborne organic pollutants (Wania & MacKay, 1999; Wania *et al.*, 1999). Recent modelling work has included forests into multimedia models (McLachlan & Horstmann, 1998; Wania & McLachlan, 2001). The role of plants in deposition of pollutants to forest ecosystem has been investigated by analysing litter-fall (Isidorov & Jdanova, 2002; Wenzel *et al.*, 2002). Wenzel et. al. (2002) have studied chlororganic pollutants in litter-fall and upper layers of soil. The global distribution of some organochlorines in rural soils have recently been mapped (Meijer *et al.*, 2003). Because plants is the first step in terrestrial food webs the role of plants in transfer of airborne organic pollutants from plant to man or animal have been investigated in the chain pasture-cow-man in Europe (McLachlan, 1996) and recently in an Arctic food chain with lichen and caribou as base (Kelly & Gobas, 2001).

Methods

Sampling and material

In this work needles from Scots pine, (*P. sylvetris)*, were used for all studies except for some samples in the geographical material of Paper II. Three mountain pines, (*P. mugo*) growing at different locations, were sampled to get a picture of the accumulation in a species having a higher number of year classes compared to Scot pine and also to compare the uptake of different species. There are advantages using conifers in general and Scots pine in particular for accumulation studies of airborne pollutants. Scots pine is widely distributed in the Northern Hemisphere, which makes it possible to sample on a large geographical scale. The evergreen foliage with needles of different growth year present also simultaneously makes them suitable to studies on a seasonal basis. Scots pines have usually three year classes simultaneously in central Europe, but at northern latitudes and at high altitudes they can have up to six or more year-classes. Compared to for instance Norway spruce (*Picea abies*) the year classes of Scots pine are easily distinguishable, making them an easier to handle as study objects. Scots pines have also been used for monitoring purposes for a long time (Eriksson *et al.*, 1989; Jensen *et al.*, 1992; Strachan *et al.*, 1994).

Samples were collected at edges of stands and at heights over 1.5 m (except for the methodology study in (Paper I) from trees of different age, but mostly from rather young individuals (15-20 years) because of the difficulties in sampling full grown pines with the lowest branches high above ground.

In the Paper I, samples were collected at a small peninsula, Ören near Nynäshamn, Sweden. Needles were collected from the southern, western, northern and eastern edge around the shore and from two stands with different density in the centre of the peninsula. Four trees were sampled from different heights.

The material in Paper II was sampled along a north-south transect from Northern Scandinavia to Southern Germany in May-June and an additional material from eastern Europe was sampled in August the year after (Figure 3).

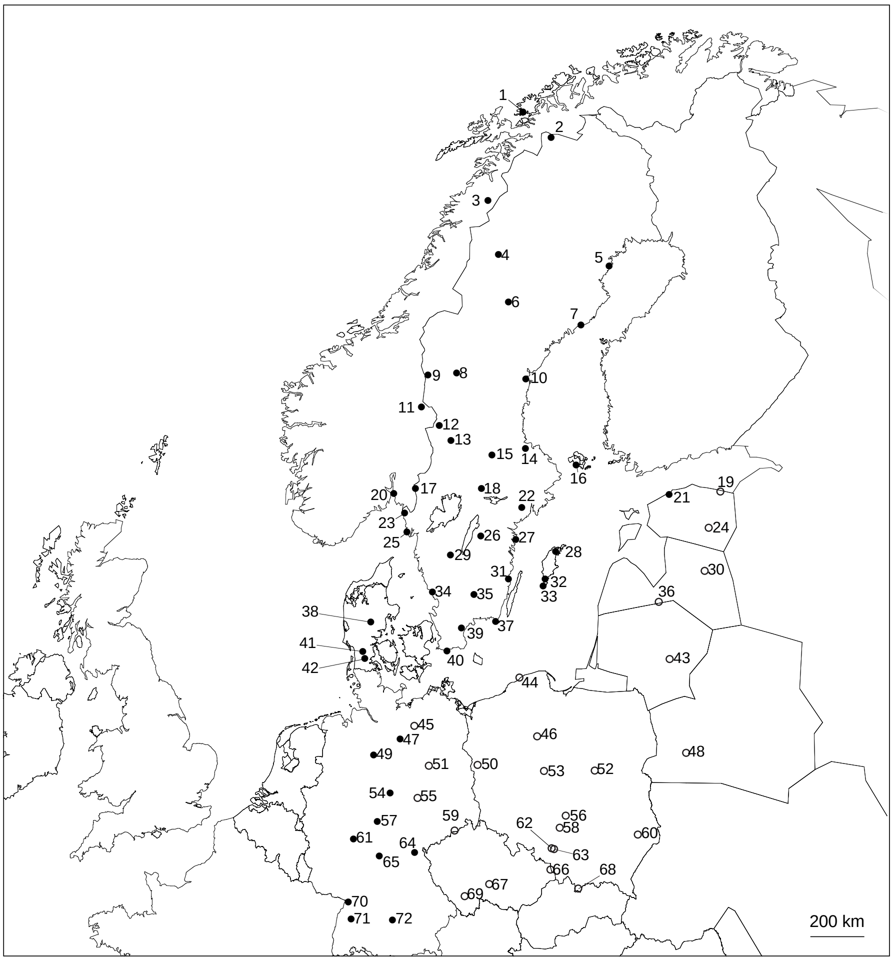


Figure 3. Sampling location and geographical co-ordinates for analyse of POPs in pine needles ● sampled in 1989 and, ○ sampled in

The material for study of seasonal variation of a number of airborne organic compounds (Paper III) was collected just off the campus area of Stockholm University. The sampling for analysis of seasonal variation in HCHs and terpene content was made at the Ultuna campus of the Swedish University of Agricultural Sciences, Uppsala.

Except for Paper I, samples for analysis of airborne organic pollutants was pooled from several trees and/or different part of single trees into batch samples, and the replicates were of the same batch sample. The intention was to make an intensive study of the same material and not as a study of the biological variation. In the study of sampling methodology (Paper I) sampling of different trees gave no significant differences. Some of the explanation for this may be that this study was performed in a planted forest with probably only one or a few provenances of pines. Thus, the biological variability within that forest may have been relatively small.

Organochlorines

To be able to analyse organic pollutants in needles it is necessary to extract the soluble cuticle lipids in which they are sorbed. The extraction time and solvent used varies in different studies of airborne organic pollutants in needles (Reischl *et al.*, 1987; Safe *et al.*, 1992; Wenzel *et al.*, 1997; Weiss, 1998). Fairly long extraction times can be needed to extract all soluble cuticular lipids. Needles of spruce (*Picea abies*) needed 6 hours in dichloromethane to extract all soluble cuticular lipids (Riederer, 1989). Kylin et. al. (1996) concluded that intact fresh needles needed to be extracted for 48 hours to get reproducible results of pollutant concentration.

Selective solvent extraction methods have been developed to separate different compartments of the cuticle (Reischl *et al.*, 1987; Hauk *et al.*, 1994; Umlauf *et al.*, 1994; Kylin *et al.*, 1996; Wenzel *et al.*, 1997) produced an extract of the outer wax by extracting with dichloromethane 10 minutes and an inner fraction by extracting n-hexane 2 x 10 minutes respectively and called the inner fraction potentially bioavailable. But the ultrasonic method used to obtain the outer extract might destroy cellmembranes increasing the risk of extracting the internal lipids. Successively increased extraction times of intact spruce needles were used to show different diffusion behaviour of airborne organic pollutants (Reischl *et al.*, 1987; Umlauf *et al.*, 1994). The longest extraction time used (100 min) was not sufficient to extract the smaller compounds HCHs and chlorobenzenes, while the larger compounds such as PCBs and DDT were extracted quantitatively. Kylin & Sjödin (2003) made extracts of the remaining material after 48 hours extraction of fresh pine needles achieving the pollutants associated with internal lipids (or very hard bound to cuticular material). Discriminating between epi- and intracuticular fractions by solvent extraction is not possible as the solvents penetrate the hydrophobic cutin and dissolve some or most of the soluble lipids embedded in the cutin (Riederer, 1989; Riederer & Markstädter, 1996). Jetter et. al. (2000) compared two mechanical methods for removal of the epicuticular wax with solvent extraction. They concluded that even a short chloroform extraction and or the solvent used when applying a polymer film in one of the mechanical methods was sufficient to extract some of the intracuticular lipids.

In this work the extraction and cleanup methods from Kylin et al (1994; Kylin *et al.*, 1996) have been used with various modifications. In short intact fresh needles were extracted in dichloromethane for 48 hours. The extracts were partitioned with an aqueous solution of *ortho*-phosphoric acid (0.1 mole/l) and sodium chloride (0.9 % weight:weight) and passed through a silica gel and silica gel:concentrated sulphuric acid column. In the seasonal study (Paper II) the extracts were further fractionated by HPLC on a nitrophenyl silica column to obtain reproducible results of PCB concentrations. The organochlorines were analysed on a gas chromathograph equipped with an electron capture detector, sometimes using dual columns in parallel.

In the methodology study (Paper I) HCHs, HCB, and DDT were analysed, and in the geographical transect (Paper II) DDE and DDD were also included. In the study from Stockholm on seasonal accumulation (Paper III) also the six PCB congeners CB 28, CB52, CB138, CB153, CB170, and CB180 were analysed. In this study the air levels were measured in parallel with the sampling of needle. The filters and the PUF plugs were extracted in dichloromethane and treated in same way as the needle samples. In Paper IV, the HCHs were the only airborne organic pollutants analysed

Terpenes

The traditional way of extracting terpenes from plant material is steam distillation (Chalchat *et al.*, 1985; Hawthorne *et al.*, 1988; Muzika *et al.*, 1990; Roussis *et al.*, 1995; Orav *et al.*, 1998). This is also the method used to achieve the essential oil used for different commercial purposes. More recent solvent extraction (Muzika *et al.*, 1990; Cool *et al.*, 1991; Cool & Zavarin, 1992; Rafii *et al.*, 1996), supercritical fluid (SFE), and other advanced extractions have also been tested (Hawthorne *et al.*, 1988; Reverchon *et al.*, 1995; Orav *et al.*, 1998). Some have used solid phase extraction of the most volatile substances (Fäldt, 2000; Lagalante & Montgomery, 2003) and the interests for such methods will probably grow. A comparative study of solvent extraction and SFE gave equally results. With SFE is it possible to get a more specific extraction and compared to steam distillation of conifer needles. Using the correct modifier, SFE has e.g. been shown to be more efficient in extracting sesquiterpenes and oxygenated terpenes (Orav *et al.*, 1998). In this study (Paper IV), we needed a fast non-laborious method to be able to extract many samples. Therefore a method using solvent extraction with pentane modified from Cool (1991) was used.

Intact needles were analysed to investigate the terpene concentration in the surficial parts of the needles, and cut needles to see the variation in total terpene content. Seven pines were analysed during 16 month

In short, cut or intact needles were extracted in n-pentane using octadecane as internal standard. The extracts were analysed on a gas chromatograph equipped with a flame ionisation detector.

Base of calculation

Concentrations of persistent hydrophobic pollutants in samples of animal tissues are often calculated on a lipid weight basis. Because of their hydrophobic character the analytes will be partitioned between in different tissues according to their lipid content. Lipid content of the tissues and foliage area are considered the most important plant species specific properties influencing uptake in vegetation (Simonich & Hites, 1995b). Some studies has suggested that pollutant concentrations and rate constants should be normalised to the lipid concentration of the vegetation or its surface area, especially when directly comparing different species and tissues (Schreiber & Schönherr, 1992; McCrady, 1994; Simonich & Hites, 1994). A comparison of using dry weight or lipid weight as basis for PAH concentrations in a number of plant parts, among them pine needles, during the growing season showed that the lipid concentration had a large effect on PAH concentration, especially in needles (Simonich & Hites, 1994).

However, using the needle (or leaf) lipid content as base for calculation is complicated. A substantial part of the endogenous hydrophobic compounds, 'lipids' in a wide sense, are either volatile and can disappear during evaporation steps, or are not extractable at all with any solvent (non-destructive methods). There is also limited knowledge about how different types of hydrophobic compounds in the needles may affect the uptake and accumulation of airborne organic pollutants. The best base to use is probably the area, but this is not easily measured in large materials of sampled needles. The area has however shown to be well correlated to the length of needle (Flower-Ellis & Olsson, 1993) which in turn is linear correlated with dry weight (Flower-Ellis pers. com). Fresh weight is also well correlated to dry weight if the needles are water saturated before weighing.

For simplicity, the dry weight was used as base of calculation in this work. However, there are problems associated with the use of the dry weight as well. Photosynthesis starts in late winter or early spring as soon as the climatic conditions allow. To avoid wasting this photosynthetic activity the pines start producing starch. This starch is stored in needles until the growing period begin and can constitute more than 30% of dry weight (Flower-Ellis & Olsson, 1993). To overcome this complication, the starch content must be subtracted from dry weight to have a comparable calculation base all year around. Alternatively, if the approximate starch variation is known, this can be taken into account when interpreting the data.

Result and discussion

Sampling methodology (Paper I)

Paper I addresses the first question asked when starting a field study: How do we take representative samples? Samples were collected in different compass direction around a small peninsula and at different heights from 4 trees at each compass direction. The total variation of DDT, α-HCH, γ-HCH and HCB concentrations was low in the whole stand. HCB was significantly lower at the lowest sampling height and DDT concentration was significantly lower in the denser stand in the centre of the peninsula. A possible reason for lower levels of DDT in a denser stand is capacity for partitioning to particles in air. Any plausible explanation for the lower level of HCB, compared to the other compounds, could not be given.

The outcome was that one should sample at the edge of stands and avoid sampling lower than 1.5 meter from ground.

Geographical variations (Paper II)

Interestingly, the levels of α-HCH are similar throughout the material from central, northern and eastern Europe, probably reflecting that the use of this chemical had ceased at the time of sampling. The origin of α-HCH was probably distant sources of emission where technical lindane was still used. The increasing concentration of γ-HCH in samples further south and in the east reflects that lindane was still in use in some parts of Europe. DDT and DDE levels also reflected old and newer use of this chemical. HCB showed no north-south trend but instead increased levels near urban areas, which is an expected pattern of a compound nowadays associated with industrial activities.

An interesting finding in this study was an increased concentration of all airborne organic pollutants in older needles in the whole material disregarding how many year classes available on the tree. Only some of the oldest needle especially those sampled in August contained lower levels of pollutants compared to younger needles. This was assumed to be a result of the senescence process and is confirmed by the seasonal study showing a decrease in the oldest year class in the end of growing season (Kylin & Sjödin, 2003).

The geographical pattern of airborne organic pollutants in pine needles reflected more or less an expected emission pattern. This confirms that it is possible to map local and regional sources and perhaps catch a decreasing trend of banned compounds, which also is shown in previous papers (Eriksson *et al.*, 1989; Jensen *et al.*, 1992; Weiss, 1998). However, this has to be done on a large geographical material to get background levels and on long-term sampling to be able see any trends in time. When studying time trends it is important to be aware of the accumulation in needles during their lifetime, and that starch accumulation during part of the year may affect the interpretation. Comparing geographical material one has to take into account that both abiotic and biotic factors affecting the uptake can differ between sites and climates. The use of pines for monitoring is limited to comparing concentration levels relative other levels in space and time. The use of pines, or other plants, to determine air concentrations is not realistic with the knowledge of today. There are too many factors causing variations in concentrations in pine needles not related to air concentrations.

Seasonal variations (Papers III and IV)

Concentration of all analysed compounds except the highest chlorinated PCB congeners, CB-170 and CB-180, increased in the surface wax during the needles whole life time in the long-term study of accumulation in pine needles in Stockholm (Paper III) (Figure 4). The compounds expected to exist mainly in gas phase (the HCHs, HCB, CB-28, and CB-52), did not decrease at any time of the whole sampling period, taking the raised starch content in spring and early summer into account, meaning that no net revolatilisation took place in the long-term perspective.

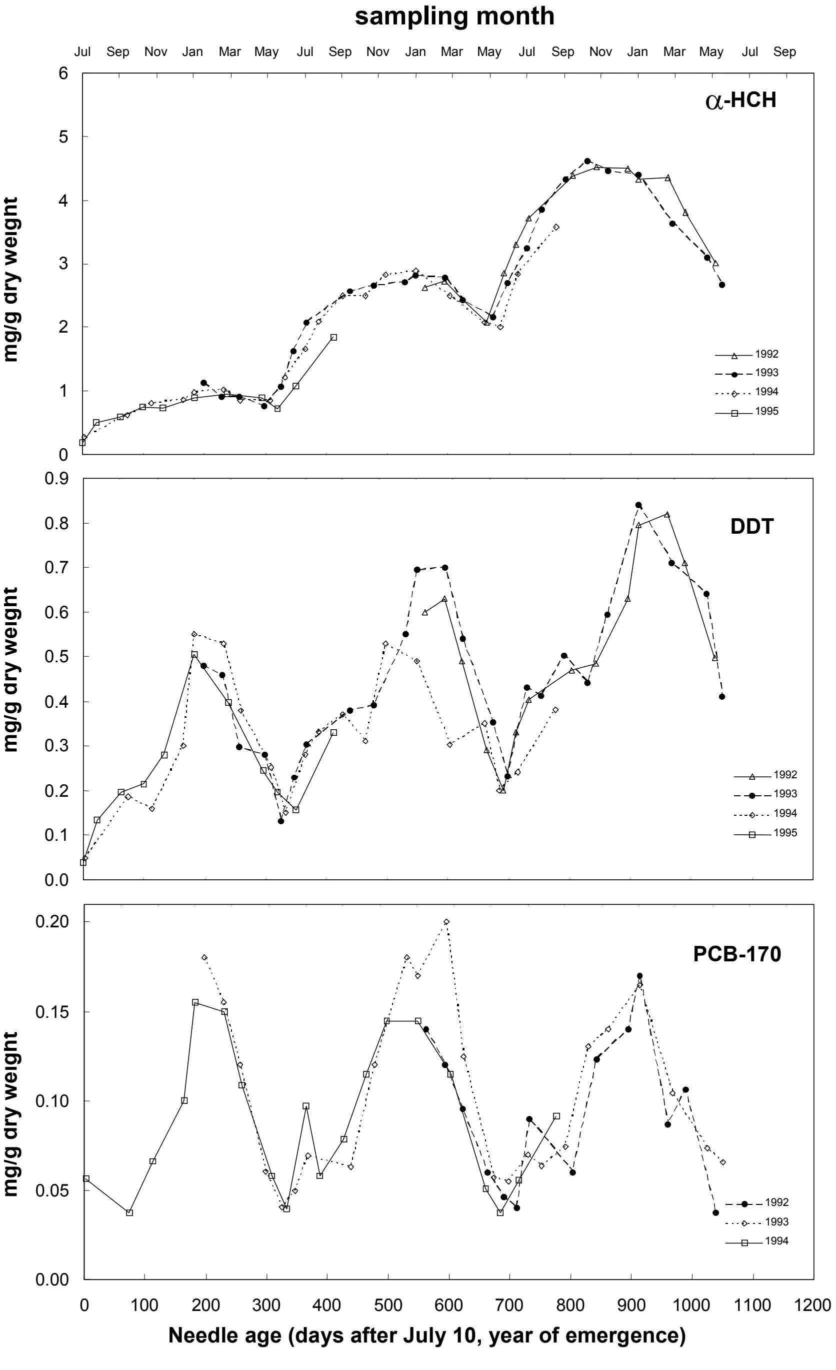


Figure 4. Concentration of organochlorine in wax extracts of Scots pine needles from the day of emergence. Three to four year classes analysed.

The compounds expected to be partly bound to particles in air (DDT, DDE, CB-138, and CB-153) showed both an increased accumulation rate in spring/summer and a peak during the cold period. For the heptachlorinated CB-170 and CB-180 only an accumulation peak in autumn/winter was detected.

These results and the results presented earlier from the same material (Kylin & Sjödin, 2003) indicate that the accumulation during the life span of the needles is dominated by a gaseous uptake. The particle bound deposition takes place in autumn and are probably temperature dependent, but the gaseous accumulation most be explained by some other factor. The partitioning coefficient between octanol air (K_{OA}) has been proposed to be most useful properties in predicting the behaviour of organic compounds in the interaction between cuticle-air (Tolls & McLachlan, 1994; McLachlan, 1999). Non-equilibrium between plant and air has been found for many compounds (McLachlan *et al.*, 1995; Schreiber *et al.*, 1996). However, compounds with low K_{OA} are expected to reach equilibrium rather fast and not accumulate in plants according to theoretical models (McLachlan, 1999). To this class belongs HCB, α-HCH and γ-HCH which are those showing the highest total accumulation.

The most striking feature in the accumulation pattern of gaseous compounds is the high uptake rate in summer. From the data available it is obvious that this seasonal variation in the accumulation pattern can be caused neither by a temperature dependent condensation/deposition process, nor by a change in air concentrations. Air levels of α-HCH were approximately the same during the whole sampling period, γ-HCH showed peaks in air levels during spring giving only a small effect on concentrations in needles (Figure 4). Obviously, there is some factor increasing the accumulation rate of the needle during spring/summer. Most probably there is some biological factor regulated by the vegetation period changes the uptake rate of gaseous airborne organic compound.

It has been proposed that some biological factor to cause the high uptake rate of gaseous compounds in spring/summer by changing the hydrophobic capacity of the needles. The question is what biological factor or factors can account for this change in hydrophobic capacity. Mono-and sesquiterpenes are small volatile hydrophobic compounds released from many plant species during summer. In a gravimetrical analysis of the extractable material from surface of pine needles the content of total soluble lipids increased up to 12 % of dry weight in summer compared to 2 % in winter (Kylin *et al.*, 2002). At least some of this material was mono- and sesquiterpenes. Terpenes are hydrophobic compounds expected to have a seasonal variation. Recently the biological functions of terpenes in plants have gained increased interest. It has been shown that monoterpenes play a role in the thermotolerance of the photosynthetic apparatus (Delfine *et al.*, 2000; Singaas, 2000). It is therefore reasonable to assume that the production and levels of terpenes is increased during hot and dry conditions during the vegetation period as compared to the winter period. This was the reason to study a possible correlation between terpene content and accumulation a gaseous organic airborne pollutant.

Terpenes

The most interesting result from the parallel study of terpenes and HCHs is the increase during summer of all terpenes in extracts of intact needles in contrast to the total extracted terpene content from cut needles. This shows that terpenes for some reason are present in the surface wax to a higher degree during the warm season than during the cold. Irrespective of the amounts of terpenes in the internal tissues of the needles, an increase of terpenes in the cuticle during the summer may affect both the air-plant partitioning of airborne organic pollutants, and also the diffusion rate through the cuticle.

There are many factors causing considerable variation in content and composition of terpenes. In this study large variation between trees was seen and an unexpected fast production of high terpenes levels in single samples suspected to be caused by some stress factor. We also saw a diurnal variation and indication of influence of weather. Other possible factors not addressed here are geographical differences, variation between species and there are even reports on difference in mono/sesquiterpene ratio in different part of needles (Bernard-Dagan, 1988).

There is, however, a continuous accumulation or increased release of terpenes in the outer layer of the needle during the warm period combined with an increased HCH concentration during the needles lifetime. This supports the theory that terpenes might affect the lipid layer of needles and enhance the accumulation of hydrophobic compounds. As a matter of curiosity can be mentioned that when we stored terpene standards in tubes with caps lined with a thick membrane, probably of some silicon-based material, we noted that the membranes swelled to more than the double size and the inner PTFE membrane was in many cases broken. With no other parallels drawn between plant cuticles and silicon based membranes the “gas phase” of terpenes seems to be able to affect material in their surroundings.

General Discussion

The outer part of the cuticle consisting of cuticular waxes is thought to be rate-limiting for the uptake of hydrophobic organic compounds. (Schreiber *et al.*, 1996). We suggest that the biologic factor responsible for the accumulation pattern of gaseous compound in pine needles are suggested to be something enhances the uptake by affecting this barrier. Some organic compounds have the capacity to mediate transport of agrochemicals through this barrier by changing the viscosity of the wax (Schönherr & Baur, 1996). To explain the accumulation during the life span of the needles, the mediated transport of organic pollutants into the cuticle must be combined with a high storing capacity for those compounds holding them in the needles although they are fairly volatile as far as the compounds studies here go. The cuticle is asymmetric this has been shown by Riedrer and Schönherr (1989) by an experiment with isolated cuticle membrane, and with the polymeric membrane after the soluble lipids have been removed. The fenoxi acid 2,4-D was absorbed into the membranes and the efflux rate through both the outer and inner layer of the membranes was measured. The rate through the inner layer of the cuticle membrane was 50-80 times higher than through the outer layer and for the polymeric membrane up to 6 times higher. This asymmetry of the cuticular transport may give a lower diffusion rate out from the needle compared to the diffusion into it. In effect this would 'trap' the compound inside the needles. Another explanation is a large storage capacity of the cuticle and resin ducts combined with a low diffusion out and low probability of revolatilisation during the cold period.

There are, of course, large differences between the properties of the gaseous HCHs and agrochemicals applied to the surface of a leaf in an aqueous solution. But the information we get from those studies might tell us something about the properties of the cuticle that can help us understand the behaviour of organic compound in cuticle and thereby the accumulation pattern we observed.

The hydrophobic character of this rate-limiting layer, consisting mainly of soluble lipids, makes it plausible to be affected by small hydrophobic compounds solved into it or passed through it. Therefore terpenes and other volatile organic compounds can be suspected to change the uptake capacity of this wax layer.

The cuticle is a heterogeneous membrane and the uptake of organic compounds into the cuticle is considered, by many researchers to be biphasic and that a two-compartment is needed to model the uptake (Hauk *et al.*, 1994; Tolls & McLachlan, 1994; McLachlan *et al.*, 1995; Schreiber *et al.*, 1996; Schönherr & Baur, 1996). The biphasic uptake is proposed to consist of a rapid adsorption equilibrium with the outer layer of the cuticle and a slower diffusuion into the cuticle observed both with isolated cuticles (Schreiber *et al.*, 1996), uptake – clearence studies (Hauk *et al.*, 1994; Tolls & McLachlan, 1994) and field studies (Hung *et al.*, 2001; Kylin & Sjödin, 2003). For many airborne organic pollutants and plants it have been shown that equilibrium will not be reached during the whole experiment, the whole growing period of some studied plants (Hauk *et al.*, 1994; Tolls & McLachlan, 1994; McLachlan *et al.*, 1995), and, as have been presented the total lifetime of foliage (Kylin & Sjödin, 2003). It is however difficult to mechanically separate the compartments responsible for this. Kylin and Sjödin (2003) have shown an accumulation in internal lipids of needles, by further extraction of what is left in needles extracted with the method above (Figure 5). This accumulation is seemingly linear in contrast to the non-linear accumulation in the more easily extracted lipids.The difference in accumulation kinetic in extracts of the internal of needles might indicate the presence of a third compartment in needles.

An accumulation during the growing period have been indicated also in other plant species than conifers (Hung *et al.*, 2001). A field study showed accumulation of SOC independent of air concentration in grass of the genus *Holcus* during six weeks (Hung *et al.*, 2001). Accumulation of airborne organic pollutants during in both evergreens and annulas has been observed in some small screening studies of field samples (Appendix A). If the uptake of gaseous airborne organic pollutants in all plants is similar to the one observed in Scots pine, vegetation obviously have a great influence on the fate of those compounds in terrestrial ecosystems. Deposition of airborne organic pollutants into terrestrial environments is proved by findings in litterfall and upper layer soils of a pine forest and in rural soils on a global scale.

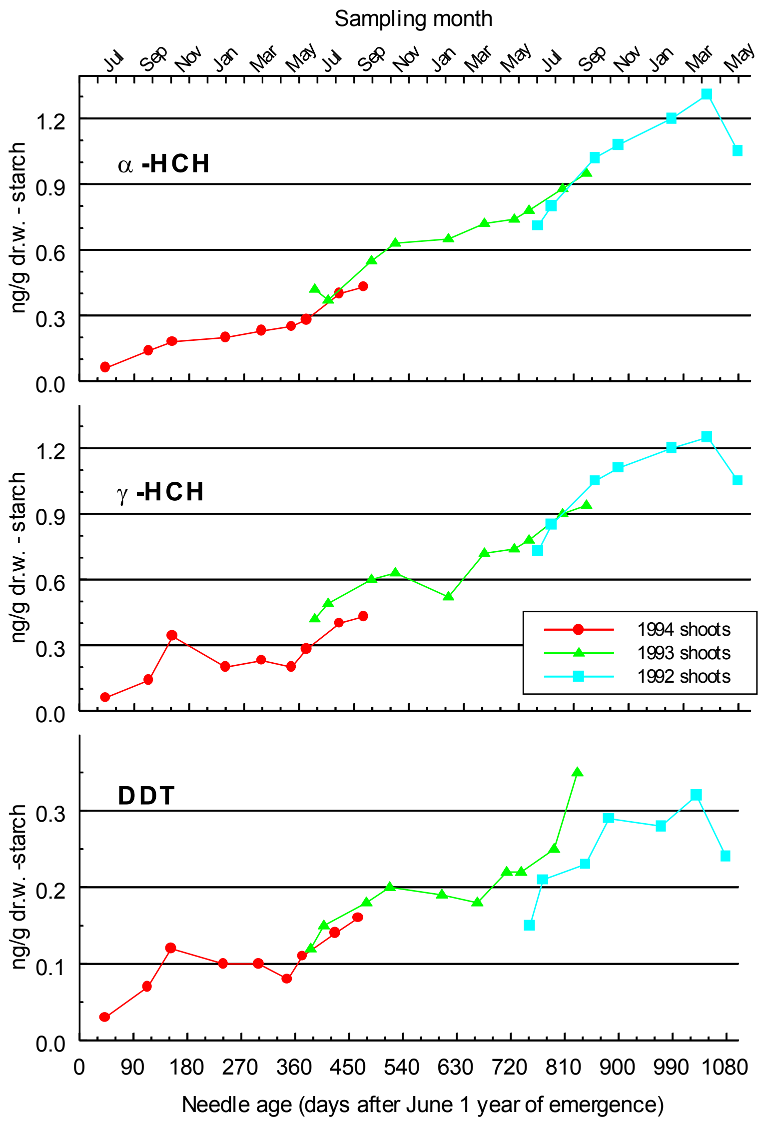


Figure 5. Concentration of organochlorines in internal lipids of three year classes of Scots pine needles from the day of emergence.

Reflection and Future research

Uptake of airborne organic pollutants in vascular plants is obviously a complicated process that we have only begun to understand. The most puzzling features are the uptake of the more volatile compounds occurs predominantly during summer, and the accumulation throughout the life span of the needles. To fully understand the processes, we propose that have more information is needed on senescence and factors that affect the hydrophobic properties of plants.

The data presented in this thesis is not the only indication that vascular plants accumulate hydrophobic compounds for a substantial time of their life span. The long series presented in Paper III, the longest that we know of on the uptake of airborne organic pollutants in foliage, indicates that accumulation continues until the start of senescence. The evidence from the large number of trees in Paper II, further backs up the conclusion that accumulation is a general rule in pines. Therefore, processes related to senescence need to be investigated to understand how these affect the uptake of airborne hydrophobic compounds.

The other big question is what factors govern the hydrophobic properties of plants. In Paper IV we have investigated the most obvious candidates. But there are other compounds beside terpenoids that may have an effect. It has been shown that several plants produce toluene when they are under stress (Zabkiewicz & and Allen, 1975; Heiden *et al.*, 1999), e.g. drought stress. Other compounds, such as xylenes and styrene, are also implicated (Robert Franich, Forest Research Institute of New Zealand, pers. comm). It is probably necessary to understand the reasons for production of these compounds and their cycling in the foliage if we are to understand the hydrophobic properties of plants. Conclusions from individual studies will be complicated, as several stress factors (drought, temperature, insect and fungal attack etc.) may trigger the production of these compounds.

A third factor of importance to understand is what structures in the foliage that have the capacity to retain even fairly volatile SOCs so that the net long-term effect is accumulation with very little obvious revolatilisation. At present we have seen that even the most volatile SOCs that are traditionally included in analyses of persistent organic pollutants accumulate in pine needles, but we know nothing about even more volatile anthropogenic compounds. Presumably, there will be an upper limit where the volatility will take precedence over the capacity of the foliage to retain the contaminants, and where a fairly rapid equilibrium between air and plant will dominate.

Future research needs to address all these issues. May of them need to be studied simultaneously in the field. The ideal project would be of long duration, have the capacity to analyse a wide range of organic pollutants in the air and foliage, and at the same time analyse factors affecting the hydrophobic properties of the plants. In the meantime we will have to manage by cross-correlating data from short projects on different aspects of plant life and the concentrations of organic contaminants in air and foliage.

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Appendix A

Plants screened for accumulation of SOCs in the foliage

Plants screened for accumulation of SOCs in the foliage. Main target analytes were the HCHs. Only few samples were analysed and no measure of statistical uncertainty can be given.

Evergreens
For evergreen foliage each year-class was tested separately. In all cases older foliage had higher levels of HCHs than younger foliage.

Conifers
Monterey pine, *Pinus radiata*, 3 year-classes
Norway spruce, *Picea abies*, 6 year-classes
Silver fir, *Abies alba*, 8 year-classes

Angiosperms
Holly, *Ilex sp*., 2 year-classes
Rhododendron, *Rhododendron sp*., 2 year-classes
Yucca, *Yucca sp*., 3 year-classes
White Arctic Bell-Heather, *Cassiope tetragona*, 15 year-classes. The first year-class was analysed separately, the other year-classes were analysed two together.

Annuals

Oak, *Quercus robur*.
Analysis of leaves started when the leaves have expanded fully. Samples were taken every week. Two sets of leaves appear each summer with 5-6 weeks between bud burst for the first and second set of leaves. When both old and young leaves were present older leaves had higher levels of HCHs than the younger leaves. Accumulation in both sets of leaves for 6 weeks until the older leaves were attacked by mildew.

Beech, *Fagus sylvatica.*
Analysis of leaves started when the leaves have expanded fully. Samples were taken every week. Accumulation during the eight weeks the sampling continued.

Maize, *Zea mays*
As for other grasses new leaves appear during growth period until the flowers are set. Analysis started when the first leaves had expanded and successively new sets of leaves were included. Accumulation was occurred for approximately six weeks of each set of leaves before they started to turn yellow. With three sets of leaves the older leaves held higher concentrations than the younger.

Henrik Kylin, unpublished data.