Atmospheric deposition of semivolatile organic compounds to plants

Atmosferische depositie van semi-vluchtige organische verbindingen op planten

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de Rector Magnificus prof. dr. H.O. Voorma
ingevolge het besluit van het College voor Promoties
in het openbaar te verdedigen op

donderdag 28 september 2000 des namiddags om 12.45 uur

door

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geboren op 5 juli 1970 te Rotterdam

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The research described in this thesis was financially supported by the Inspectorate of Health Protection of the Dutch Ministry of Public Health, Welfare and Sports and was carried out at the Transport Physiology Research Group of the Faculty of Biology and at the Research Institute of Toxicology, both at Utrecht University.

ISBN 90-393-2462-X

Cover design: Femke Bulten and Martine Bakker

De eekhoorn en de mus wandelden op hun gemak door het bos en spraken over allerlei dingen die in hun gedachten opkwamen. De zon scheen en zij hadden niets te doen.
‘Wat is onmeetbaar?’ vroeg de eekhoorn.
‘Ja,’ zei de mus, ‘dat is bijvoorbeeld hoe warm de tijd is of wie er het meest van de lucht

De mus legde aan de eekhoorn uit hoe je de diepte van de slaap kon meten. ‘Ik heb dat zelf ontdekt,’ zei hij, en sloeg enkele bescheiden stofjes van zijn vleugels af. Toevallig passeerden zij juist de tor die onder de eik lag te slapen en zacht snurkte. ‘Let maar op,’ zei de mus. Hij nam tien stappen van de tor af en draaide zich om en zei: ‘Tor’.
Er gebeurde niets. De tor sliep door. De mus kwam één stap dichterbij en zei weer: ‘Tor’. Er gebeurde weer niets.
Zo ging hij telkens een stap dichter naar de tor toe, tot hij voor zijn oor stond en tamelijk luid riep: ‘Tor’. Maar de tor sliep door.
De mus wendde zich tot de eekhoorn en zei: ‘Hij slaapt onmeetbaar diep.’

De mus streek zijn veren glad en zei: ‘Ik ben nu aan het uitvinden hoe je kunt meten hoe diep

‘Dat zal wat zijn,’ zei de eekhoorn.
‘Ach,’ zei de mus, ‘als je wilt kun je alles uitvinden.’

Naar: Toon Tellegen - *Toen niemand iets te doen had*

Bedankt allemaal!

Aan het begin:

Twee dames hebben mij aangezet tot het AIO-schap, waarschijnlijk zonder dat zelf te weten. Daarom wil ik ze hier apart bedanken.

Else, jij maakte mij enthousiast voor onderzoek naar milieuvreemde stoffen in planten. Samen zetten we de eerste stappen op het onderzoeksvlak. Dat het goed beviel, bewijst het feit dat ze voor ons beiden uiteindelijk tot een promotie-onderzoek naar contaminanten in planten leidden. Zoals te verwachten valt van twee AIO's in hetzelfde onderzoeksgebied, hadden we vele boeiende gesprekken over de literatuur. Wonderlijk genoeg leverde de binnen- en buitenlandse fictie en fantasy de meest interessante gespreksstof op.

De tweede verantwoordelijke is Marca, die mijn afstudeeronderzoek op het RITOX begeleidde en na afloop zei dat ik "best AIO kon worden". Marca, ik hoop dat ik mijn studenten net zo enthousiast en kundig heb begeleid als jij mij. Zeker bij mijn eerste studenten heb ik mij vaak voor de geest gehaald hoe jij het zou aanpakken. Bedankt!

Op het lab:

Chris, dank voor je enthousiasme en inzet. Ik denk met plezier terug aan al onze besprekingen, vooral die van mijn (door jou volgekrabbelde) manuscripten. De vele discussies over taalkundige merkwaardigheden staan mij nog levendig voor de geest. Het verschil tussen de begrippen "invloed" en "effect" is me in ieder geval voor altijd duidelijk geworden.

Dick, bedankt voor het op het juiste spoor zetten van de nog groene AIO die ik was en dat ik op elk moment van de dag bij je binnen kon vallen met vragen. Van jouw aanpak - gewoon beginnen met een "rekenmodelletje"- heb ik veel opgestoken.

Johannes, toen Dick's deur jouw deur werd, bleef ie nog steeds open staan. Bedankt voor de peptalks, je telefoon-akties ("is er nog een glovebox in het land?") en het immer razendsnelle doorlezen van manuscripten.

Mijn andere milieuchemische collega's: keukenprinses-Manuela, nieuwe woorden schud ik uit mijn mouw-Gert-Jan, many great ideas-Philipp, stil water diepe grond-Rik, altijd een luisterend oor-Agnes en niet op haar mondje gevallen-Femke, wil ik bedanken voor al het gedeelde joelen (De Maagd, persoonlijke mededeling 1995) en treuren in de afgelopen jaren.

Veel dank ook aan de analytische steunpilaren. Allereerst aan Manuela, die met grote nauwkeurigheid en veel geduld de cleanup van plantenmonsters uitzocht. Judith, bedankt voor de bergen opgewerkte plantenmonsters en de gezelligheid. Door ons geklets over boeken, theater en andere interessante gespreksstof is er meer dan eens een monster verloren gegaan,

maar dat mocht de pret niet drukken. Frans, Theo en Jolanda, bedankt voor jullie hulp bij allerhande zaken.

Mijn begeleidingscommissie, bestaande uit Gijs Kleter, Henk van der Schee en Hans Jeuring, wil ik hartelijk bedanken voor de oprechte belangstelling voor het onderzoek, de opbouwende kritiek en de stimulerende discussies.

Alle mensen die de verdere randvoorwaarden voor het doen van onderzoek schiepen, in en om het Kruyt- en het Wentgebouw en Nieuw Gildestein wil ik de loftrompet toesteken. In het bijzonder wil ik de "eerste hulp bij laboratoriumbenodigdheden", bestaande uit Cor en Ed & ~~Willem-Bever~~ o nee, Gerard, noemen. Of het nou ging om gasflessen of een afwasborstel, een glovebox of de waterkoker, jullie stonden altijd paraat om ze te leveren, respectievelijk te repareren. Veel dank ook aan de afdeling Beeldverwerking & Vormgeving van Biologie en de Audiovisuele Dienst van Scheikunde voor alle mooie foto's, posters, dia's en andere plaatjes. Last but not least wil ik de mensen van de kassen, en met name Fred, bedanken, die al mijn planten kweekten en de strijd aangingen met luizen, meeldauw en konijnen. Allemaal ontzettend bedankt voor de uitstekende service en de leuke gesprekken!

Mijn studenten: Ben, Desirée, Ilco, Casper, Inge, Michel, Johanna, Lucy, Dimitry, Pieter, Berta en Bas, jullie hebben bergen werk verzet. Niet al jullie namen zijn terug te vinden in dit boekje, maar denk niet dat we er niets van hebben opgestoken. Ilja, bedankt voor je uitstekende scriptie, ik heb hem er nog vaak bij gepakt. Alle andere studenten Milieuchemie, bedankt voor de sfeerverhogende bijdragen, met name bij kerst- en andere etentjes. Ook alle andere (ex-)medewerkers van Transportfysiologie, Milieutoxicologie en alle andere RITOX-ers en BOEV-ers wil ik bedanken voor hun interesse en de gezelligheid aan de koffie- en/of borreltafel.

Many thanks to Mike McLachlan and colleagues for having me in your lab in Bayreuth. I enjoyed my stay very much and although the results of the experiment are not part of this thesis, I learned a lot about plant cleanup.

Peter, vielen Dank für die Diskussionen über Pflanzen und mittelflüchtige Verbindungen, für die Hilfe im Labor, und für das Teilen deines Hauses und deiner Freunde. Du siehst: ich hab' wirklich meine Ohren steif gehalten.

Buiten het lab:

De "(bijna allemaal weg uit) Utrecht-club": dank voor alle weken en weekeinden in huisjes, tenten en boten, waarin ik de tijd niet kreeg om over onderzoek na te denken. Het broodnodige relativeren van het promotie-onderzoek komt voornamelijk op rekening van mijn (al dan niet AIO-) vriend(inn)en: Bedankt allemaal!

Mijn paranimfen Nynke en Agnes wil ik bedanken voor het functioneren als uitlaatklep. Nynke, jij was de eerste jaren degene die het moest ontgelden als ik wat te vertellen had, maar ook na je vertrek uit het Kruyt bleef je geïnteresseerd in alle ins en outs. Agnes, je nam de rol

van Nynke probleemloos over, bovendien heb je je meermalen bewezen als adequate probleemoplosser. Meiden, bedankt!

Mijn familie en ook die van Fokke wil ik bedanken voor de interesse in mijn werk (“hoe is het met je planten?”) en het vertrouwen in mijn kunnen.

Mijn ouders, Ben en Lizelot, jullie hebben me niet alleen vrij gelaten, maar ook altijd gesteund in mijn keuzes. Bedankt!

Fokke, jij had het hele proces al eens meegemaakt en zoals altijd was je mijn favoriete mentor (lees schipper). Bedankt voor het dekschrobben, het laveren bij tegenwind, en me op tijd kielhalen wanneer ik weer eens zat te stressen. Dat we nog lang de “fok bak” mogen zetten en zo vele obstakels (lees skûtsjes) kunnen omzeilen.

Contents

1

Introduction

Introduction

There is a growing attention for the deposition of semivolatile organic compounds (SOCs) to vegetation (Simonich and Hites 1995a). SOCs are hydrophobic organic chemicals, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), with vapour pressures roughly between 10^{-6} and 10^{1} Pa (Bidleman 1988). These compounds originate from various human activities (and also from natural sources) and are of special interest because of their global distribution, persistence, high tendency to bioaccumulate and known or suspected toxicity.

In the 1980s, it was demonstrated that pine needles and plant leaves accumulate gaseous SOCs from the ambient air (Buckley 1982, Gaggi *et al.* 1985, Reischl *et al.* 1987, Eriksson *et al.* 1989, Frank and Frank 1989). The contamination of vegetation with SOCs causes concern, as plants are at the base of the food chain, and therefore a starting point for transfer of these compounds to animals and humans (Figure 1). This is of major importance for the exposure of the human population to SOCs in industrialised countries. For PCBs and PCDD/Fs, the exposure occurs particularly via the pathway atmosphere → vegetation → cattle → milk/ dairy/ beef → humans (Fürst *et al.* 1990, Theelen *et al.* 1993, Fries 1995, McLachlan 1996, Duarte-Davidson *et al.* 1997). In the case of PAHs, the consumption of cereals, oils and fats and leafy vegetables is more important (Dennis *et al.* 1983, Edwards 1983).

A second point of interest is that vegetation serves as a sink for SOCs and in this way cleanses the air (Simonich and Hites 1994a, Duarte-Davidson *et al.* 1997, Wagrowski and Hites 1997). At first, it was estimated that 44% of the PAH emissions in the northeastern United States was removed from the atmosphere by vegetation (Simonich and Hites 1994a). However, after sampling at more locations, this estimate was revised downward to 4% (Wagrowski and Hites 1997). Forests are shown to be effective air filters for organic chemicals (Matzner 1984, Horstmann and McLachlan 1998), at least for the very hydrophobic compounds, with relatively high octanol-air partition coefficients ($\log K_{oa}$ values between 7 and 11). This range includes a large number of PAHs, PCBs and PCDD/Fs (McLachlan and Horstmann 1998).

Naturally, plants can only act as a temporary sink, since they live only for a limited period of time. The fall of dead leaves and twigs (Matzner 1984, Horstmann and McLachlan 1996) and the erosion or shedding of wax layers caused by wind, rain and/or rubbing of the leaves (Chamberlain 1970, Horstmann and McLachlan 1996) all result in a deposition of SOCs to the soil (Figure 1). In addition, there is some evidence that, after deposition to plant surfaces, some SOCs (PCDD/Fs) are photodegraded by sunlight (McCrady and Maggard 1993, Schuler *et al.* 1998). However, this could not be confirmed experimentally by other researchers (Welsch-Pausch *et al.* 1995).

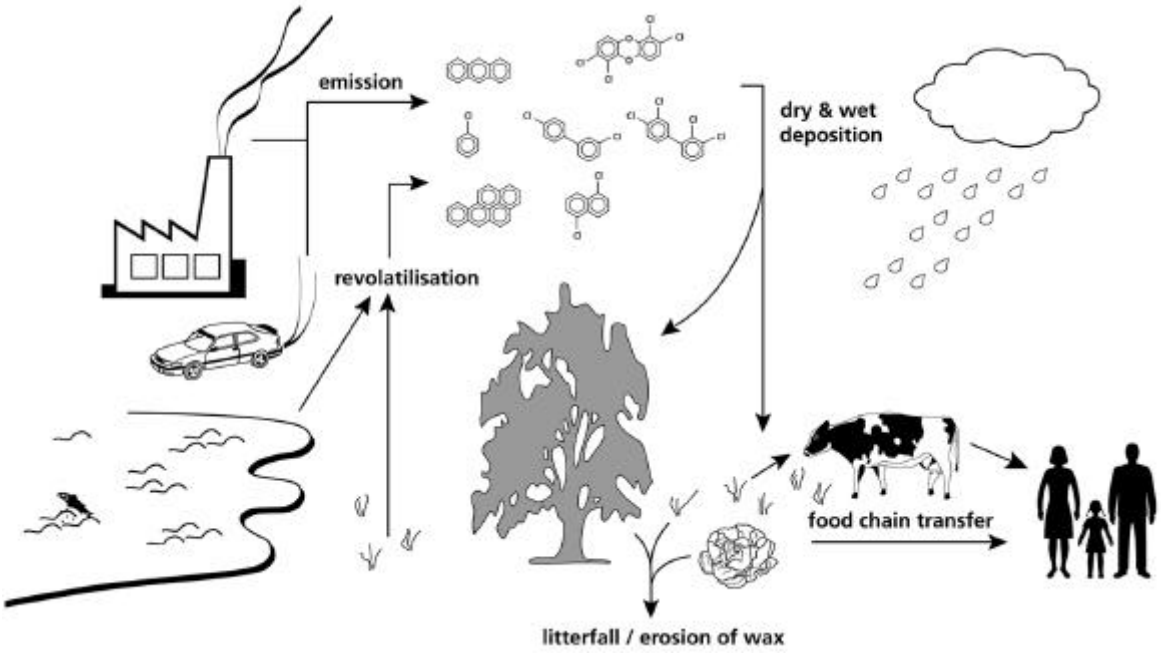

Figure 1. Some atmospheric SOCs, their sources and their deposition to plants and entrance into the food chain

Finally, the deposition of SOCs to vegetation gets attention because plants are being used as biomonitors for air pollution (Kylin *et al.* 1994), as vegetation integrates contamination over time and plant samples are much easier to collect than air samples. Plants can be used to identify point sources and to qualitatively determine regional and global contamination levels of organic pollutants (Simonich and Hites 1995b). For example, by comparing plant samples from all over the world, evidence was found for the global distillation (or grasshopper) effect; *i.e.* the tendency of compounds to move through the atmosphere from relatively warm source regions to colder, higher latitudes, where condensation occurs (Calamari *et al.* 1991, Simonich and Hites 1995b).

Scope of this thesis

As mentioned above, SOCs are a concern for human health and they can enter the food chain via atmospheric deposition to plant surfaces. The deposition of SOCs to plants depends on (1) the physicochemical properties of the compounds (such as vapour pressure and hydrophobicity), (2) the environmental conditions (temperature, wind and rain) and (3) the characteristics of the plants (lipid content, leaf morphology), (Paterson *et al.* 1990).

In order to assess the risk of the consumption of SOC-contaminated vegetables, meat and dairy to humans, predictive models are developed (*e.g.* Trapp and Matthies 1995). These models estimate the concentrations of these compounds in plants from given concentrations in the air. A number of plant characteristics, such as the lipid content, surface area to volume ratio and growth rate, are used in these models. Nevertheless, risk assessment is often performed with values for a "standard" plant, instead of plant specific values. The objective of this research is to investigate whether this is justified, or if plant specific parameters should be employed when SOC concentrations, resulting from atmospheric deposition, are estimated in plants. Therefore, the effect of the some compound properties and some plant characteristics on the atmosperic deposition of SOCs to plants is investigated in this thesis.

The following questions are addressed:

- What is the role of particle-bound versus gaseous deposition of SOCs?
- Which plant characteristics play a role in the deposition of SOCs to plants?
- Where are deposited SOCs localised in the plant?
- Is it possible to use concentration ratios in risk assessment, *i.e.* predicting concentrations in one plant species from known concentrations from another?
- How should plant characteristics be included in predictive models?

Thesis outline

In **Chapter 2** a literature review on the atmospheric deposition mechanisms of SOCs and the factors that influence these processes is presented. The experimental setup of this research was to analyse SOCs in different plant species after exposure to the compounds in the field (PAHs) and in the laboratory (PAHs and chlorinated hydrocarbons). Firstly, the extraction of cuticular wax of two test species (*Plantago major* and *Lactuca sativa*) was studied (**Chapter 3**). To obtain realistic deposition data, semi-field experiments were carried out by placing *Plantago* species in an "open greenhouse" and in a field plot in the Botanical Gardens of Utrecht University (**Chapter 4-6**). To examine gaseous deposition under controlled laboratory conditions, a flow-through system was developed to expose the plants (**Chapter 7**). A case study was performed in a contaminated area in Zelzate, Belgium (**Chapter 8**). In **Chapter 9**, two predictive models are evaluated with respect to plant characteristics. Finally, the results of this research are summarized and discussed (**Chapter 10**).

2

Atmospheric deposition of semivolatile organic compounds to PLANTS: a review

Adapted from:
Martine Bakker, Johannes Tolls and Chris Kollöffel. In: *Persistent, bioaccumulative and toxic chemicals I: Fate and exposure*, Lipnick, R.L., J.L.M. Hermens, K.C. Jones, D. C. G. Muir, Editors. American Chemical Society Symposium Series, 2000, Chapter 16, p. 218-236.

Abstract

Atmospheric SOCs can be deposited to plants by (dry) gaseous and (wet and dry) particle-bound deposition. Once deposited on the leaf surface, a compound can be transported through the cuticular wax layers to the inner compartments of the plant. The transport rate depends on the molar volume of the compound and the tortuosity of the waxes. The deposition of SOCs depends on the physicochemical properties of the compound, the environmental conditions and the plant characteristics. The most important compound property is the octanol-air partition coefficient K_{oa}. With increasing K_{oa}, the dominating deposition process goes from equilibrium partitioning to kinetically limited dry gaseous deposition to particle-bound deposition. Temperature and wind are important environmental parameters. Under equilibrium conditions, temperature has a large influence on the plant-air partition coefficient K_{pa}. The effect of temperature in non-equilibrium situations is less pronounced, because it takes time for concentrations in plants to change, particularly for compounds with high K_{oa}'s. Wind can increase the transfer of the chemical from the air to the plant surface, by increasing the turbulence and decreasing the thickness of the laminar boundary layer. Differences between SOC concentrations in different plant species are often fairly small (< a factor of ~8), but sometimes also large differences (up to a factor of >50) are measured. Under equilibrium conditions, the differences in SOC concentrations may be explained by the amount and composition of the lipids. In cases where equilibrium has not been approached, the age of the leaves, the leaf area and the plant architecture have been shown to be key factors in determining the SOC concentrations in plants.

Introduction

In this chapter the mechanisms and kinetics of atmospheric deposition of semivolatile organic compounds (SOCs) to plants are reviewed and the factors influencing the deposition process are discussed. The deposition of organic compounds in plants has been previously reviewed by Paterson *et al.* (1990), McLachlan, (1995) and Simonich and Hites (1995a). The present review includes more recent findings and pays attention to the role of plant characteristics.

In the first part of this review, the important pathways of deposition of SOCs to plants, namely particle-bound and dry gaseous deposition, are presented. Once deposited to the leaves or stems of the plant, the compound can be transported through the cuticle, the lipid-like layer which forms the interface between the atmosphere and the plant. The transport through this layer is adressed in the second part of this chapter. The final part deals with a number of important factors that influence the deposition of SOCs to plants: the octanol-air partition coefficient (K_{oa}) of the compound, the temperature and the characteristics of the plant.

Uptake of SOCs from the soil via the roots of the plant will not be addressed in this review, because the contamination of aerial plant parts with SOCs occurs primarily via the atmosphere. As SOCs are very hydrophobic and therefore have low water solubilities, the concentrations in soil water are low. Furthermore, the dissolved compounds, which are taken up by the roots, partition to the membrane lipids in the root epidermis. Therefore, the availability of these compounds for translocation to the shoots is low and this route is negligible for most plants (McCrady *et al.* 1990, Paterson *et al.* 1990, Schroll and Scheunert 1992, Wild and Jones 1992, Wang and Jones 1994). The only exceptions are plants of the family of *Cucurbitaceae* (zucchini, pumpkin), which may produce root exudates that promote translocation of SOCs (Ecker and Horak 1994, Hülster *et al.* 1994).

Little research has been done on toxicity of SOCs to or degradation in plants. Most of these studies investigated these processes with cell cultures. Hence, little is known about the relevance of these processes in intact plants. For these reasons, neither toxicity nor degradation will be discussed in this chapter.

Pathways of SOC deposition

Deposition of atmospheric SOCs to plants occurs via several pathways. Because SOCs partition between the gas phase and atmospheric particles, a major division can be made between gaseous and particle-bound deposition. Since the solubility of the hydrophobic SOCs is very low in rain droplets or other precipitation, wet deposition of gases is of minor importance (Duinker

and Bouchertall 1989, McLachlan and Horstmann 1998). Compounds bound to atmospheric particles can reach the plant surface by both dry and wet deposition.

Uptake of compounds that are volatilized from highly contaminated soil is another pathway, particularly in more basal aerial plant parts (Trapp and Matthies 1997). Also, contaminated soil particles can be transported directly to the plant surface by wind or splash (Jones and Duarte-Davidson 1997, Trapp and Matthies 1997).

The deposition of airborne compounds, whether they are gaseous or particle-bound, to plant leaves involves three steps (Figure 1).

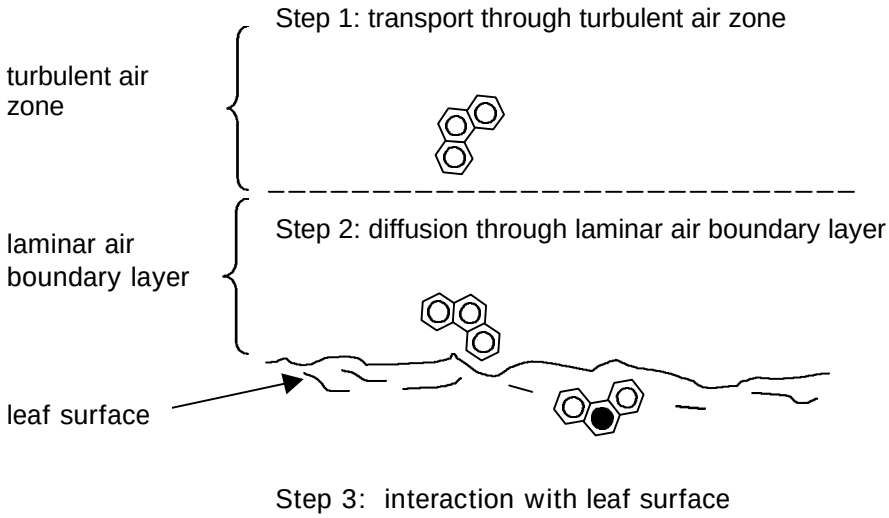

Figure 1. Schematic representation of the three steps in the deposition process of hydrophobic organic compounds from the atmosphere to a leaf.

First, the contaminant is transported from the (turbulent) atmosphere to the laminar air boundary layer surrounding the leaf. Subsequently, the contaminant has to be carried across the boundary layer. In this layer the airflow is parallel to the leaf surface, and the wind speed is highly reduced but increases with distance from the surface (Gregory 1961). While in the "bulk" atmosphere contaminants are transported convectively by turbulent wind eddies, gaseous compounds and particles < 0.1 µm (Davidson and Wu 1989) can only be carried through the laminar boundary layer by Brownian motion, which is 3 to 7 orders of magnitude slower than transport under turbulent conditions (Jones 1983). The final step in the uptake process is the interaction of the compound with the leaf surface. Deposited particles may simply adhere to the leaf surface, react with it chemically, or bounce off if the surface is smooth (Davidson and Wu 1989). For gases, the third step comprises the adsorption to the surface or diffusion into the cuticle (Schreiber and Schönherr 1992).

The different pathways are all a function of (1) the physicochemical properties of the compound (such as vapour pressure, hydrophobicity, molecular weight), (2) environmental

characteristics (temperature, wind) and (3) plant characteristics (surface area, lipid content and composition, architecture of the plant), (Paterson *et al.* 1990).

Particle-bound deposition

The particle-bound fraction of SOCs in the atmosphere depends on the ambient temperature, the available particle surface and the compound's volatility (Pankow 1987). Recently, Finizio *et al.* (1997) proposed the compound's octanol-air partition coefficient (K_{oa}) to govern the gas-particle distribution, with high K_{oa} values favoring high particle-bound fractions.

The particle size distribution of PAHs has been intensively studied and shows that PAHs are largely bound to particles < 1-2 µm (Venkataraman and Friedlander 1994, Poster *et al.* 1995, Schnelle *et al.* 1996, Chen *et al.* 1997a, Chen *et al.* 1997b, Kaupp and McLachlan 1999). Data for other SOCs of interest are scarce. Nevertheless, also PCDD/Fs were shown to be primarily associated with small particles (Kaupp and McLachlan 1999). However, although the SOC loading of the large particles is low, dry particle deposition fluxes to the earth's surface appear to be dominated by large particles, since their deposition velocity is relatively high (Holsen *et al.* 1991, Kaupp and McLachlan 1999). In contrast, wet deposition of particle-bound PCDD/Fs was reported to be dominated by fine particles (Kaupp and McLachlan 1999).

Whether dry or wet deposition is the most important route of particles to plant surfaces is primarily dependent on the amount of precipitation. In a coastal environment, wet deposition is likely to be of more importance in supplying SOCs than at an inland site (McLachlan 1995, Jones and Duarte-Davidson 1997). Several studies indicate that the total amount of particle deposition to the earth's surface or to large water bodies is dominated by wet deposition (Swackhamer *et al.* 1988, Schröder *et al.* 1997, Kaupp and McLachlan 1999). Nonetheless, considering the deposition of particles to plant surfaces, the role of wet deposition is not very clear. On one hand, results indicate that rain and hail increased plant concentrations (Kaupp 1996), but on the other hand precipitation may cause a wash-off of already deposited material (Wedding *et al.* 1975). In the remainder of this review we will therefore consider wet and dry particle deposition as a whole.

SOCs may be transferred from the deposited particles to the cuticle, which has been studied with wash-off experiments. Washing of lettuce with water removed a considerable amount of the high molecular weight (MW) PAHs, but little of the small PAH phenantrene, indicating that only the latter, gaseous PAH was sorbed in the cuticle (Larsson and Sahlberg 1982). In contrast, rinsing maize leaves with aqueous solutions could only extract a minor part of the high MW PCDD/Fs and PAHs, suggesting that the compounds were desorbed from the particles or that the particles were encapsulated in the cuticle (Kaupp 1996). While this remains

inconclusive, we will use the term uptake only to refer to the dry deposition of gases, whereas this term will not be used for the deposition of particle-bound SOCs.

Dry gaseous deposition

Mechanism

The uptake of gaseous SOCs involves passive diffusion of the compounds between the atmosphere and the cuticle of the plant. The cuticle is an extracellular, non-living, lipid layer which forms the interface between the atmosphere and the plant and protects the plant from desiccation and from fungal and insect attack (Eglinton and Hamilton 1967), (Figure 2). It is usually characterised by the presence of two specific classes of lipids: soluble waxes and insoluble polyester cutins (Holloway 1994). Recently, it has been proposed that also another major lipid polymer, cutan, is present in the cuticular membrane (Jeffree 1996). Waxes occur embedded in the cuticular membrane (intracuticular waxes) and lying across the surface of the membrane (epicuticular waxes). Waxes consist of complex mixtures of long-chain aliphatic and cyclic components, including primary alcohols, alkanes, esters, fatty acids and triterpenoids (Eglinton and Hamilton 1967, Baker 1982, Holloway 1982, Kirkwood 1987). The cuticle is not homogeneous, but composed of a number of layers of which the characteristics vary according to species, age of the plant (Kirkwood 1987) and environmental conditions (Cape and Percy 1993).

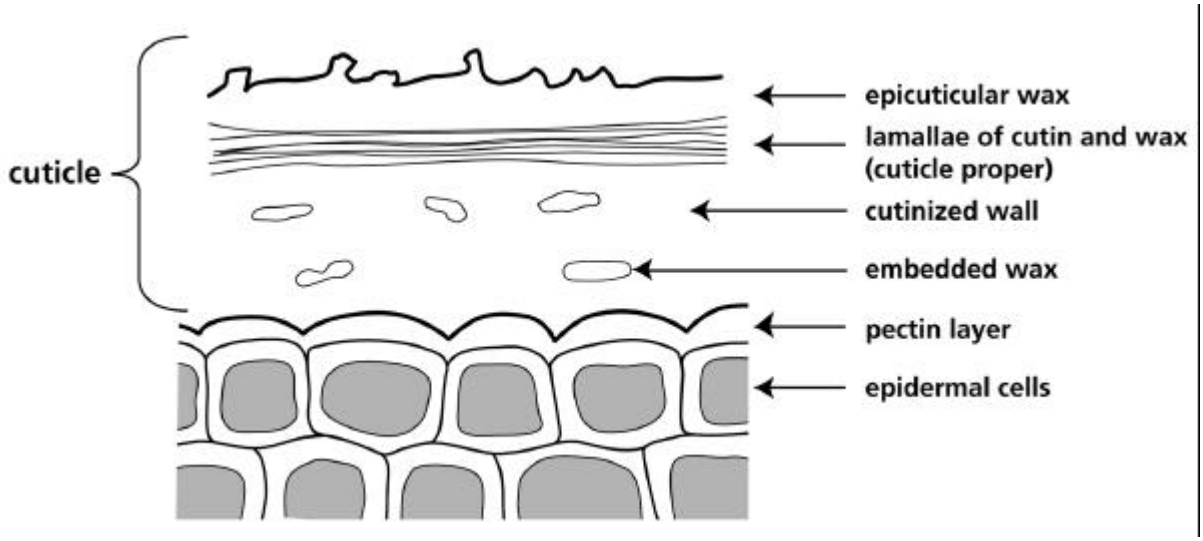

Figure 2. Schematic representation of the cuticle of a plant

Uptake of SOCs via stomata is probably not important. They will prefer to enter the cuticle, hence the contribution of the stomatal pathway will be negligible to that of the cuticle (McLachlan 1999). Moreover, there is some evidence that the cuticle extends farther along the cell walls that form the epidermal boundary of the substomatal cavity (Martin and Juniper 1970). So, even after entering the stomata, SOCs can still be sorbed to these "internal" cuticular waxes.

Partitioning process

The diffusion of gaseous SOCs into the cuticle can be viewed as a chemical partitioning process between the gas phase and the vegetation, in which a compound is transferred from the atmosphere to the plant until an equilibrium has been reached (Paterson *et al.* 1990, McLachlan 1995). Relationships have been found between experimentally determined air-to-plant concentration factors (K_{pa}) and the octanol-air partition coefficient (K_{oa}), (Travis and Hattemer-Frey 1988, Bacci *et al.* 1990a, Tolls and McLachlan 1994, Simonich and Hites 1994b and 1995a, Kömp and McLachlan 1997a). The K_{oa} of a compound can be estimated by dividing K_{ow} by the dimensionless Henry's Law constant. In addition, direct measurements have been performed and for an increasing number of SOCs (PCB's, chlorinated benzenes, PAHs) measured K_{oa} values at different temperatures are now available (Harner and Mackay 1995, Kömp and McLachlan 1997b, Harner and Bidleman 1998).

In many experiments a high correlation between $\log K_{pa}$ and $\log K_{oa}$ is demonstrated (Table 1), which means that the air-to-plant lipid and air-to-octanol free energies of phase change are similar. When the slope of a plot of $\log K_{pa}$ versus $\log K_{oa}$ is equal to one (as in Tolls and McLachlan 1994, see Table 1), K_{pa} and K_{oa} are linearly related and the lipid fraction of the plant behaves as octanol. As a consequence, under equilibrium conditions, accumulation of SOCs in vegetation can be described as

$$K_{pa} = L \cdot K_{oa} \qquad (1)$$

where L is the lipid fraction of the plant (Paterson *et al.* 1990, McLachlan *et al.* 1995).

However, $\log K_{pa}$-$\log K_{oa}$ plots for a number of plant species give slopes different from one. In a field experiment, a slope of the log-log plot for PAHs in needles, leaves and tree bark of 0.48 was observed (Simonich and Hites 1994b and 1995a), while the slope for PCBs in a field pasture amounted to ~0.4 (Thomas *et al.* 1998), (Table 1). Kömp and McLachlan (1997a) and Böhme *et al.* 1999), studied uptake of SOCs in different plant species and found slopes varying considerably between species (Table 1). The large differences between the slopes were attributed to the differences in lipid quality (Kömp and McLachlan 1997a). Another explanation for shallow slopes of the log-log plots is a deviation from equilibrium, leading to greater underestimations of K_{pa}'s of the compounds with higher K_{oa} values. However, equilibrium conditions were checked in all experiments, except for the study into PAH concentrations in

needles, leaves and tree bark by Simonich and Hites (1994b). A slope smaller than one indicates that the lipophilicity of octanol is higher than that of the vegetation and vice versa. Hence, when slopes deviate from one, octanol is not a good descriptor for plant lipids (Kömp and McLachlan 1997a).

Table 1. Slopes and correlation coefficients of plots of $\log K_{pa}$ (or $C_{vegetation}/C_{gaseous\ phase}$) versus $\log K_{oa}$ from several studies.

plant	*compounds*	*study*	*slope*	r^2	*reference*
azalea	a, b, c	lab	0.91[e]	0.85	(Paterson *et al.* 1991)
ryegrass	b, c, d	lab	1.00[e]	0.95	(Tolls and McLachlan 1994)
needles, leaves seeds, tree bark	d	field	0.48[e]	0.98	(Simonich and Hites 1994b)
pasture	b	field	0.32-0.47	0.66-0.96	(Thomas *et al.* 1998)
ryegrass	b	lab	1.15	0.98	(Kömp and McLachlan 1997a)
clover	b	lab	0.70	0.86	(Kömp and McLachlan 1997a)
plantain	b	lab	0.87	0.98	(Kömp and McLachlan 1997a)
hawk's beard	b	lab	0.74	0.97	(Kömp and McLachlan 1997a)
yarrow	b	lab	0.57	0.93	(Kömp and McLachlan 1997a)
ryegrass	b, c, d	field	0.60[f]	0.93	(Böhme *et al.* 1999)
creeping thistle	b, c, d	field	0.62[f]	0.96	(Böhme *et al.* 1999)
dandelion	b, c, d	field	0.78[f]	0.98	(Böhme *et al.* 1999)
plantain	b, c, d	field	0.65[f]	0.96	(Böhme *et al.* 1999)
yarrow	b, c, d	field	0.35[f]	0.72	(Böhme *et al.* 1999)
lady's mantle	b, c, d	field	0.53[f]	0.95	(Böhme *et al.* 1999)
sunflower	b, c, d	field	0.39[f]	0.85	(Böhme *et al.* 1999)
autumn hawkbit	b, c, d	field	0.77[f]	0.97	(Böhme *et al.* 1999)
white clover	b, c, d	field	0.66[f]	0.90	(Böhme *et al.* 1999)
corn	b, c, d	field	0.57[f]	0.90	(Böhme *et al.* 1999)

a: pesticides, b: PCBs, c: chlorobenzenes, d: PAHs e: K_{oa}'s calculated instead of measured, f: calculated with the nine compounds having the lowest K_{oa} values

One- or two-compartment model

Bacci and coworkers (Bacci and Gaggi 1987, Bacci *et al.* 1990a, Bacci *et al.* 1990b) perormed controlled uptake and clearance experiments with azalea and a series of organic compounds. The results were interpreted using a simple one-compartment linear first order model of diffusive exchange, in which the leaf behaves as a well-mixed homogeneous compartment. This model is described by the following equation:

$$\frac{dC_p}{dt} = k_1 \cdot C_a - k_2 \cdot C_p \qquad (2)$$

in which C_p and C_a are the concentration in the plant and in the air ($mol \cdot m^3$), respectively, t is time (h), and k_1 (h^{-1}) and k_2 (h^{-1}) are the uptake and elimination rate constant, respectively. When $t \rightarrow \infty$, equilibrium is approached and the ratio of C_p and C_a is equal to K_{pa} ($= k_1 \cdot k_2^{-1}$). This model was used to describe uptake of SOCs (under controlled conditions) in spruce needles (Reischl *et al.* 1989), grass (McCrady and Maggard 1993), and in a number of different other plant species (both foliage and fruits, McCrady 1994). Trapp and Matthies (1995) and McLachlan (1999) presented a more detailed one-compartment model for the atmospheric deposition of organic chemicals to foliar vegetation:

$$\frac{dC_p}{dt} = k_1 \cdot C_a - k_2 \cdot C_p = \frac{A \cdot g}{V} \cdot C_a - \frac{A \cdot g}{V \cdot K_{pa}} \cdot C_p = \frac{A \cdot g}{V} \cdot (C_a - C_p / K_{pa}) \qquad (3)$$

where A is the surface area of the vegetation (m^2), g is the conductance (or deposition velocity; $m \cdot h^{-1}$), V is the volume of the vegetation (m^3) and K_{pa} is the air (gas phase) to plant partition coefficient ($m^3 \cdot m^{-3}$).

Assuming A/V, g, K_{pa} and C_a to be constant, and $C_p = 0$ at $t = 0$, equation (1) can be integrated to give

$$C_p = K_{pa} \cdot C_a \cdot (1 - \exp\left[- \frac{A \cdot g \cdot t}{V \cdot K_{pa}} \right]) \qquad (4)$$

Note that some of these parameters are dependent on environmental conditions (*e.g.* g and K_{pa}). As these are mostly not constant in time, this assumption is not always justified.

Several other researchers tried to fit their uptake and clearance data into a model and concluded that a one-compartment model failed (Schreiber and Schönherr 1992, Schreiber and Schönherr 1993, Hauk *et al.* 1994, Tolls and McLachlan 1994, Keymeulen *et al.* 1995). In the two-compartment model proposed by Tolls and McLachlan (1994), the leaf consists of a small surface compartment, reacting fast to changing air concentrations, connected to a larger reservoir compartment. The model described the experimental data well (Hauk *et al.* 1994, Tolls and McLachlan 1994).

Whether a model with one or two compartments is preferable to describe plant uptake, depends on the timescale of the experiment. For most purposes the one-compartment model will suffice to describe the data, although it is clear that the assumption that a leaf is one homogeneous compartment is not fulfilled. In experiments in which short-term kinetics are studied, an additional surface compartment may be needed to accurately describe the concentration changes in the plant.

Transport through the cuticle

Designation of the compartments

Although the model fits showed that a leaf consists of more than one compartment, it is not clear which parts of the leaf actually represent these compartments. It has been proposed that in needles compounds first adsorb to the surface and subsequently partition into the cuticle (Schreiber and Schönherr 1992). Also, parts of the needle interior, namely membrane lipids and essential oil (mainly composed of lipophilic terpenoids) were thought to act as a reservoir compartment (Keymeulen *et al.* 1995).

In experiments in which needles and leaves were separated in a leaf wax and inner compartment by means of fractionated extractions, the presence of small SOCs in the inner compartment was demonstrated (Reischl *et al.* 1987, Hauk *et al.* 1994). As compounds can move through the cuticle exclusively by molecular diffusion, their mobility is inversely proportional to their molar volume (Schreiber and Schönherr 1993, Schreiber 1995, Baur *et al.* 1996, Schreiber *et al.* 1996, Baur *et al.* 1999). So only the small compounds could reach the inner compartments. Reischl (Reischl *et al.* 1987) proposed that the compounds crossed the cuticular waxes (surface compartment) to accumulate in the cutin or possibly the interior of the needle (reservoir compartment). A high sorption capacity of cutin was also pointed out by Riederer and coworkers (Riederer and Schönherr 1984, Riederer and Schreiber 1995).

Tortuosity of cuticular waxes

The thickness of the cuticle can vary from tenths of micrometers to more than ten micrometers (Price 1982), while the thickness of the epicuticular wax layer is estimated as 10% of the cuticle thickness (Bauer and Schönherr 1992). According to Fick's Law, the time needed to diffuse through a layer depends on the thickness of the layer. However, diffusion through cuticles seem independent of the thickness (Norris 1974, Price 1982). Because extraction of the cuticular waxes from the cuticle increased the mobility of the compounds by one to three orders of magnitude, waxes are considered the main transport barriers in the cuticle (Kerler and Schönherr 1988, Bauer and Schönherr 1992, Riederer and Schreiber 1995). Therefore, the composition and arrangement of cuticular waxes is of more importance for the permeability of the cuticle than the cuticle thickness.

The structure of cuticular waxes consists of two distinct phases: an amorphous and a crystalline phase (Riederer and Schreiber 1995, Kirkwood 1999). Diffusion (and also partitioning) of compounds only takes place in the amorphous, and not in the crystalline phase (Riederer and Schreiber 1995, Baur *et al.* 1996, Schreiber *et al.* 1996). The impermeable wax

crystals reduce the mobility of compounds in the wax in two ways: by decreasing the available volume and by creating a tortuous pathway. Consequently, the density of the crystals (the crystallinity) and the arrangement of the crystalline waxes determine the transport rate through the waxes (Schreiber *et al.* 1996).

The crystallinity of wax layers is relatively constant in the temperature range 5-40°C (Baur *et al.* 1999). Although this range is similar to the range of ambient temperatures, temperatures of leaves exposed to sunlight can be much (up to 24°C) higher than the ambient temperature (Baur *et al.* 1997). It appears that at higher temperatures the aliphatic crystallinity of the waxes decreases (Merk *et al.* 1998). This leads to a loss of size selectivity of the waxes, and hence, to a higher mobility of large compounds.

The tortuosity of cuticular waxes (the ratio of path length in intact cuticles and de-waxed cuticles) may vary greatly between different plant species: *e.g.* from a factor of 28 (*Citrus grandis)* to 759 (*Ilex paraguariensis*). This leads to estimated path lengths (tortuosity * 10% of cuticle thickness) ranging from 7 to 410 µm for these plants (Baur *et al.* 1999). An average SOC has a molar volume of ±150 cm^3/mol (calculated with McGowan's characteristic volumes Abraham and McGowan 1987). This leads to a diffusion coefficient of ± 10^{-16}-10^{-17} m^2/s (extrapolated from Schreiber and Schönherr 1993, Schreiber 1995, Schreiber *et al.* 1996), which is around the magnitude of the diffusion coefficients in solid materials (Schönherr and Riederer 1989, Bauer and Schönherr 1992, Riederer and Schreiber 1995, Baur *et al.* 1996). With Fick's Law it can be estimated that it takes this SOC 68 hours-28 days to cross the *Citrus* and 27–270 years to cross the 410 µm *Ilex* cuticular waxes to reach the cutin polymer. So, although cutin is known to be an effective storage compartment for SOCs, in the case of plant leaves having highly crystalline waxes, compounds may never reach this compartment because the lifetime of a leaf may be too short.

Factors influencing atmospheric deposition to vegetation

Influence of K_{oa} on deposition pathway

Dry gaseous versus particle-bound deposition

First estimations showed that each of the three deposition pathways: dry gaseous, dry particle and wet deposition, contributed to the concentrations of SOCs measured in spruce (Umlauf and McLachlan 1994c). Later, SOC concentrations in plants in outdoor locations and in greenhouses which contained filtered or non-filtered air were compared, which led to more detailed information. For a number of chlorinated pesticides and a range of PCBs in spruce

(Umlauf *et al.* 1994a) and for tetra- to hexachlorinated PCDD/Fs in ryegrass (Welsch-Pausch *et al.* 1995), dry gaseous deposition was found the principal deposition pathway. Results for ryegrass indicated that for hepta- and octachlorinated PCDDs (having high K_{oa} values), the contribution of large particles may be important. This was confirmed by a similar experiment with native grassland cultures (Welsch-Pausch and McLachlan 1996). In this experiment, dry particle bound deposition clearly played an important role for PCDD/F congeners with 6 and more chlorine atoms, while for lower chlorinated congeners dry gaseous deposition was dominant.

Nakajima and Kaupp (1996) concluded from field experiments that also for high K_{oa} PAHs (and also high K_{oa} PCDD/Fs, Kaupp 1996) the dry deposition of particles is a significant pathway for deposition to vegetation.

Three dominant deposition pathways

A number of models describing or predicting deposition of atmospheric SOCs in plants have been presented in the literature (Simonich and Hites 1995a). Nevertheless, validation studies for these models are scarce. The fugacity model for dry gaseous deposition to ryegrass, developed by Tolls and McLachlan (1994), which was modified into a one-compartment model, was validated with a field experiment. The predicted and measured values were in very good agreement (McLachlan *et al.* 1995).

In a recent publication, McLachlan (1999) used the results of this field validation to propose a mathematical framework which helps to interpret SOC measurements in plants by identifying the dominant deposition process for a given compound. In the framework (as in the field validation) three dominant processes are distinguished. The first process, equilibrium partitioning, is dominating for compounds with low K_{oa} values. Uptake of these substances is therefore linearly related with K_{oa} (Figure 3, McLachlan 1999).

In the second process, the uptake is still dominated by dry gaseous deposition, but it is independent of the compound's K_{oa} (Figure 3). This can be explained by the extremely high storage capacity of the vegetation for the chemical. As a consequence, the time needed to approach equilibrium is longer than the exposure time, so uptake of these compounds is kinetically limited (McLachlan *et al.* 1995). Particle-bound deposition, the third process, is controlling for high K_{oa} compounds. A higher K_{oa} leads to a higher the particle-bound fraction in the air and this leads to a higher contribution of particle-bound deposition (Figure 3).

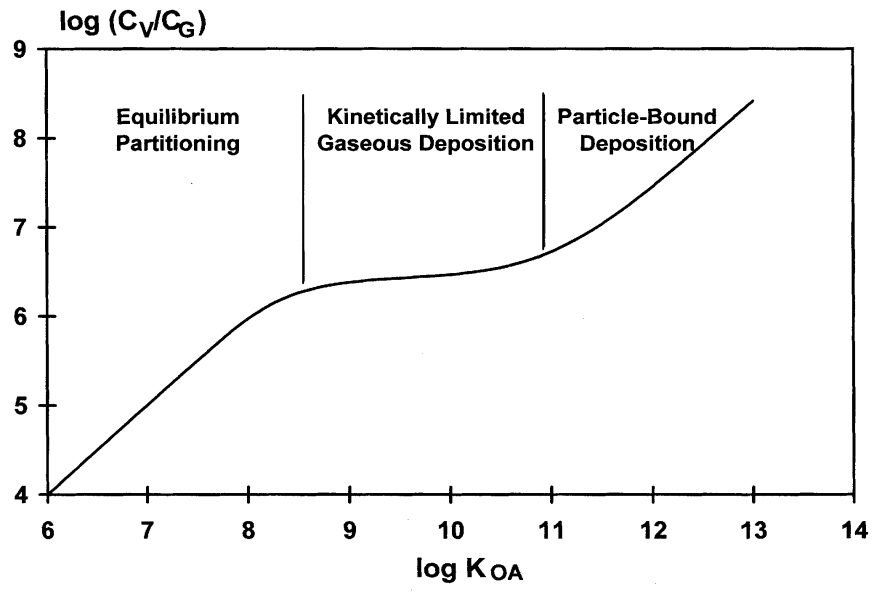

Figure 3. Illustrative plot of log $C_{vegetation}/C_{gaseous\ phase}$ vs log K_{oa} (McLachlan 1999, copyright American Chemical Society).

This framework was applied by Böhme *et al.* (1999) to analyze SOCs in ten different plant species collected simultaneously at a semirural site in Central Europe and to identify which of the three processes caused the accumulation of a given compound. The compounds from 5 different families fit nicely into the framework curves for the ten plant species, which demonstrates that the interpretive framework is the first model to successfully cover the whole scope of atmospheric deposition of SOCs to plants. The behaviour of the PAHs was different in some cases, probably due to their different gas-particle distribution from that of other compound classes (Böhme *et al.* 1999).

Compounds of which the deposition is kinetically limited, will probably never reach an equilibrium with the plant, since most leaves do not live long enough (McLachlan *et al.* 1995). This is in agreement with a field study, in which concentrations of chlorinated organic compounds in spruce needles, were still increasing after 5 years (Jensen *et al.* 1992). This information is relevant for food studies, where food crops are investigated, which are only present on the field for a relatively short time period. In these cases, equilibrium may not be reached for the high K_{oa} compounds.

However, Thomas *et al.* (1998) have shown that PCBs do reach equilibrium with grass within two weeks, because concentrations in two-week, six-week and three-months old mixed grass swards were similar. The fact that these measurements were performed on windy sites near the UK coast (*i.e.* higher turbulence, smaller boundary layers), in contrast with the inland site used by McLachlan and coworkers, may explain the difference between the studies.

In another field study in the UK all PCDD/F congeners were found to be transferred with same efficiency to grass (Jones and Duarte-Davidson 1997). This may mean that the deposition of all congeners was kinetically limited, but since large PCDD/Fs are very hydrophobic, a contribution of particle deposition is to be expected. The authors considered the wet deposition possibly important in the maritime UK environment. Another explanation may be that PCDD/Fs sorb to ultrafine particles, and may behave as gases (Jones and Duarte-Davidson 1997). The simplest explanation however, is that gaseous and particle-bound deposition rates may be similar (McLachlan, 1997).

In conclusion, the compound's K_{oa} determines the dominating deposition pathway, being equilibrium partitioning, kinetically limited dry gaseous or particle-bound deposition. However, the environmental conditions, together with the plant species, determine the K_{oa} values which mark the transition from one process to the other.

Influence of temperature

The partitioning process between air and vegetation is expected to be dependent on the ambient temperature. According to the Clausius-Clapeyron relationship, a plot of ln K_{pa} vs 1/T gives a straight line with a positive slope. This means, as the ambient temperature decreases, partitioning to vegetation increases.

Kömp and McLachlan (1997c) studied the temperature dependence of K_{pa} of PCBs in ryegrass under controlled laboratory conditions in a fugacity meter. A very strong temperature dependence was found: K_{pa} decreased by a factor of 30 (some dichlorobiphenyls) to 2000 (some octachlorobiphenyls) in a temperature range between 5 and 50°C, indicating that under environmental conditions K_{pa} values are highly variable. However, simulations with the fugacity model (McLachlan *et al.* 1995) showed that for most SOCs, the ambient temperature has little effect on the concentrations in a field vegetation. As the dry gaseous deposition of SOCs with relatively high K_{oa} values is kinetically limited and equilibrium is not approached, changes in temperature will only affect concentrations in plants after longer time periods. Only for compounds with lower K_{oa} values, the plant air concentration ratio can respond fairly quickly to a change in temperature (Kömp and McLachlan 1997c).

However, in a few field studies, in which PAHs or PCDD/Fs were determined in the atmosphere and in vegetation in different seasons, the semilogarithmic Clausius-Clapeyron relationship has been confirmed (Simonich and Hites 1994b, Nakajima *et al.* 1995, Wagrowski and Hites 1998). Apparently, although the plant-air ratios may not have been genuine equilibrium ratios, they react to temperature changes fast enough to observe a seasonal trend.

Some studies even report diurnal variation of plant concentrations. The concentrations of volatile organic compounds (VOCs) in different plants varied during the day, because of the large variation in VOC concentrations in air, due to different temperatures and traffic flows during the day (Hiatt 1998). As VOCs are not very hydrophobic and have low K_{oa} values, they can rapidly adjust to varying air concentrations. In a study in a remote forested bog, the logarithm of the SOC concentrations in air was related to the reciprocal temperature (r^2 up to 0.9; Hornbuckle and Eisenreich 1996). The authors attributed the variation to strong diurnal variations in temperature, resulting in vapour adsorption and subsequent volatilisation of the compounds from the plant surfaces (Hornbuckle and Eisenreich 1996), but the surface compartments may also be capable of a fast reaction to changes in temperature.

Influence of plant characteristics

SOC concentrations in different plant species

In several studies the SOC concentrations of different plant species were compared. Buckley (1982) found an tenfold range in PCB-concentrations (ΣPCBs varying from 32 $ng \cdot g^{-1}$ dry weight to 320 $ng \cdot g^{-1}$ dry weight) in foliage from 18 plant species in New York State. Concentrations of PCBs and some organochlorine pesticides in 12 woodland species near Siena (Italy) differed by a factor 2-6 (Gaggi *et al.* 1985), while the differences in moss, lichen, beech leaves and spruce needles in south Germany were less than a factor of 3 (Morosini *et al.* 1993). For VOCs, large differences in concentrations (factor 100-400) were found between foliage from different trees and plants (Keymeulen *et al.* 1993, Hiatt 1998). In a study in Norway, lichens were compared to spruce needles. Differences in SOC concentrations ranged from a factor of 3 up to >50 (Ockenden *et al.* 1998).

This synopsis shows that the variation between different plant species can be considerable. Consequently, it is important to know which plant characteristics cause the differences in SOC concentrations in different species.

Lipid content

Differences in accumulation between different plants can be partly explained by the lipid content of the species. Normalising to the extractable lipid content decreased the differences in PAH concentrations between leaf, needle, seed and tree bark samples collected on the same site from a factor of 5 to 2 (Simonich and Hites 1994b). However, the lipid content of plants is only important in (near-)equilibrium situations, since uptake kinetics seem independent of it

(McCrady 1994). Besides, the extractable lipid content may not represent the actual storage volume, as SOCs may also accumulate in non-extractable lipid material, such as cutin. On the other hand, SOCs may not have reached the internal lipids, but these are included in the total lipid content. Therefore, the fractional volume of the cuticle to the leaf volume may be a better normalising factor (Böhme *et al.* 1999). In practice, however, this factor is difficult to estimate.

The interspecies variation in the study of Böhme *et al.* (1999) was a factor of ~30 (on a dry weight basis), for the compounds with low K_{oa} values which had approached equilibrium. However, leaving out two of the ten species (yarrow and sunflower), the variation reduces to a factor of ~5. In addition, although the authors (Böhme *et al.* 1999) argued that the variability in K_{pa}'s was not related to the lipid content, the extractable lipid content for the eight species (without yarrow and sunflower) correlate well with K_{pa} (r^2's between 0.5 an 0.8).

Lipid composition

In the study of Böhme *et al.* (1999), the SOC concentrations in yarrow and sunflower leaves were much higher than those in the other plants, even after normalisation to lipid content and volume fraction of the cuticle. This was probably caused by a very different cuticular wax composition, which was also indicated by the relatively shallow slopes of the $\log K_{pa}$-$\log K_{oa}$ plots of these plants (Böhme *et al.* 1999). These results agree with the fugacity meter study of Kömp and McLachlan (1997a) in which the slope of the $\log K_{pa}$-$\log K_{oa}$ plot of yarrow was also the lowest (see Table 1) and the K_{pa}'s of the lower chlorinated PCBs were the highest *et al.* 1999).

In Kömp and McLachlan's study (1997a), K_{pa} values were found to vary a factor of 20 for the five species. The differences in both the slopes (see Table 1) and the K_{pa}'s were attributed to differences in the composition of the plant lipids.

Hiatt (Hiatt 1998) attempted to relate the composition of the leaf cuticular wax to the concentrations of VOCs in plants. The presence of monoterpenes, a class of wax components, was held responsible for the high concentrations of VOCs found in some species (Hiatt 1998). However, the monoterpenes were the only class of wax components determined in the waxes, hence a causal relationship cannot be shown.

Plant age

The large difference found between SOC concentrations in pine needles and lichens in Norway could not be explained by the lipid contents of the plants, but it was suggested that the differences in plant ages (2 years for the pine needles and 25-50 years for the lichens) may play a

role (Ockenden *et al.* 1998). SOC concentrations in air of a few decades ago were likely higher than those in contemporary air. Hence, the older lichens may have been approaching higher equilibrium concentrations of PCBs, and are now releasing the compounds very slowly (Ockenden *et al.* 1998).

Plant architecture

Surface area

Dry gaseous deposition occurs to all surfaces of the plant and therefore the total surface area influences the uptake rate. The variability in uptake rate constants of 2,3,7,8-TCDD in four plant species was reduced from a factor of 50 to 4 when the constants were normalised to the surface area of the plant (McCrady 1994). Similarly, in the study of Böhme *et al.* (1999), a significant relationship was found (except for three species) between uptake and the surface area to volume ratios of the different plants, for those gaseous compounds that had not reached equilibrium. In theory, the best results will be obtained when the true total surface area (i.e. taking into account the relief of the leaf surface and cuticular waxes) and not the superficial surface area would be used (Schreiber and Schönherr 1992). In practice, however, the true surface area is difficult to determine.

Aerodynamic surface roughness

Particle deposition to different types of vegetation was found to increase with increasing leaf area index (surface area of leaves divided by m^2 of ground) of plants (Witherspoon and Taylor Jr. 1970, Jonas and Heinemann 1985, Heil 1988) and the density of the foliage (m^2 leaf surface$\cdot m^{-3}$ air or kg vegetation$\cdot m^{-2}$ ground), (Chamberlain 1970, Schuepp 1989). These parameters give an estimation of the aerodynamic surface roughness of the plants; a high surface roughness leads to a higher turbulence, and therefore to a higher supply of SOCs. Also, the number and size of the vegatitive elements (leaves, flowers) as a function of height has been used to estimate aerodynamic surface roughness (Davidson *et al.* 1982).

Leaf orientation

In contrast with dry gaseous deposition, in the case of particle-bound deposition only (a part of) the upper side of the leaf will be receiving the SOCs. Researchers have tried to define this receiving leaf area as being the horizontal leaf area (Böhme *et al.* 1999), since the horizontal orientation of the leaves (together with the rough hairy surface) was found to be of importance (Welsch-Pausch and McLachlan 1996). A linear relationship between the horizontal area to volume ratio and the deposition of particle-bound compounds (except for two species) was found in the study of Böhme *et al.* (1999).

Summary

Deposition pathways

In the 1980's, it was demonstrated that atmospheric deposition of SOCs rather than uptake via roots is the dominant pathway for contamination of aerial plant parts. In more recent years, important progress has been made to elucidate the relevant pathways of atmospheric deposition. Both dry gaseous deposition and particle bound deposition (wet and dry) have been shown to contribute to the deposition of SOCs. Which one is actually controlling the deposition, depends on the K_{oa} of the compound, the plant species and the prevailing temperature and wind. Under given environmental conditions, the deposition pathway to a given plant species is governed by the K_{oa}, in which the controlling process goes from equilibrium partitioning to kinetically limited dry gaseous deposition to particle bound deposition with increasing K_{oa}.

Compartments

Uptake of atmospheric SOCs in vegetation can be described with a one or two compartment model. One-compartment models, in which all lipids are lumped together, are mostly used to describe long-term atmospheric deposition, while two-compartment models give a more detailed view of the short-term diffusion behaviour of the chemical within the leaf. The first (or surface) compartment may consist of the cuticular waxes and the second (or reservoir) compartment of the cutin, although the time to reach the cutin may be very long, due to the high tortuosity of the diffusion pathway through the cuticular waxes.

Factors influencing deposition

It is clear that the K_{oa} is the most important compound property, as it determines the controlling deposition process and can predict the K_{pa} well. However, as slopes are generally different from one, lipids of most plant species have a different lipophilicity than octanol.

Temperature and wind are important environmental parameters. Under equilibrium conditions, temperature has a large influence on K_{pa}. The effect of temperature in non-equilibrium situations is less pronounced, because it takes time for concentrations in plants to change, particularly for compounds with high K_{oa}'s. Because of different wind speeds, the rate at which plant concentrations adjust to new situations can be different for different sites. Wind can

increase the transfer of the chemical from the air to the plant surface, by increasing the turbulence and decreasing the thickness of the laminar boundary layer. This illustrates the important role of the wind, although the effect of wind on uptake rate has never been studied directly.

In many studies only small differences (< a factor of ~8) between SOC concentrations in plants have been found, but sometimes also large differences (up to a factor of >50) are measured. Under equilibrium conditions, the large differences between some plants may be explained by the amount and composition of the lipids. However, it is difficult to relate the presence of certain wax components to the storage capacity of leaves. In cases where equilibrium has not been approached, the age of the leaves and the plant architecture have been shown to be key factors in determining the SOC concentrations in plants.

Concluding remark

The (sometimes) large differences between concentrations in different plant species may have consequences for the precision of predictions of SOC concentrations in food crops. Most predictive models do not take species differences into account, although some include lipid content and/or surface area to volume ratio. The same holds for estimates of the importance of vegetation as a pollutant sink. Also for biomonitoring purposes, the large variation in concentrations between species may have consequences. Special attention should be given to the choice of the plant species, which should be ubiquitous. In addition, the age of the sample leaves should be taken into account. However, the various environmental conditions under which the leaves are sampled may have a larger influence on the concentrations in plants than the plant characteristics. This makes it difficult to draw conclusions from comparison of samples from different locations. This may not be of major influence when biomonitoring is used for identifying point sources, but it seriously complicates the use of plants as indicators of regional or global contamination levels.

3

Extraction and identification of LEAF WAX of *Lactuca sativa* AND *Plantago major*

Adapted from:
Martine Bakker, Wim Baas, Dick Sijm and Chris Kollöffel, 1998. *Phytochemistry* **47**: 1489-1493.

Abstract

The composition of leaf cuticular waxes of lettuce (*Lactuca sativa*) and great plantain (*Plantago major*) was determined for studies on the uptake and bioaccumulation of SOCs. In addition, to find a suitable extraction solvent to be used in these studies, the extraction efficiency of several solvents for the cuticular wax of the plants was studied. Leaf wax of *L. sativa* consists mainly of long chain linear alcohols and minor amounts of fatty acids, while the major components of leaf wax of *P. major* are the free polar triterpene acids oleanolic acid and ursolic acid, and the linear alkanes $C_{27}H_{56}$-$C_{33}H_{68}$. The wax composition of both plants changes only slightly with leaf developmental stage. This property makes them highly suitable as test plants in studies on uptake of SOCs. The waxes of both plant species are readily extractable by chloroform, toluene and dichloromethane. A mixture of chloroform and methanol (2:1 v/v) additionally extracted internal lipids and chlorophyll and, therefore, is not suitable. The apolar solvent *n*-hexane did not extract the triterpene acids of *P. major*. However, this solvent readily extracted the relatively apolar leaf wax of *L. sativa*. Since the extraction of SOCs (also from deeper embedded wax layers) can only be efficient if all the components of the cuticular wax are removed, we recommend testing the extraction efficiency of a solvent other than chloroform or dichloromethane for each plant species beforehand.

Introduction

Since the composition, polarity, amount and structure of the leaf cuticular wax differ widely among plant species (Walton 1990), the uptake of SOCs in the leaf wax (and thus by the plant) may be plant specific. In addition, these plant properties are dependent on leaf developmental stage and growth conditions (Baker and Hunt 1981, Kolattukudy *et al.* 1981). For studying uptake rates and bioaccumulation in plants it is thus important to have information about the chemical and physical properties of the cuticular waxes.

In order to determine the amount of SOCs in the leaf cuticular wax of plants (epi- and intracuticular wax together as a single fraction), the wax has to be extracted, without extracting internal lipids from the cytoplasmic compartment. Since most cuticular waxes contain a mixture of both polar and non-polar constituents in epicuticular wax as well as in cuticular wax, solvents need to be carefully chosen if they are to dissolve all cuticular wax constituents of the plant sample (Juniper and Jeffree 1983). Chloroform is most generally used as a solvent, since it is able to dissolve almost all compounds known to occur in leaf waxes (Martin and Juniper 1970, Kolattukudy *et al.* 1981, Juniper and Jeffree 1983). Other frequently used solvents are petrol and mixtures of chloroform and methanol (Misra and Ghosh 1991). Since SOCs are hydrophobic chemicals, they are readily soluble in apolar organic solvents. For the extraction of SOCs in cuticular waxes dichloromethane is widely used (*e.g.* Wild and Jones 1992, Umlauf *et al.* 1994b, Kylin *et al.* 1996), while n-hexane (*e.g.* Bacci and Gaggi 1987, Reischl *et al.* 1987, Calamari *et al.* 1991) and toluene (Welsch-Pausch *et al.* 1995) have been used for the extraction of SOCs in homogenized plant material.

In the present study, we determined the cuticular wax composition and the extraction efficiency of several solvents for the waxes of lettuce (*Lactuca sativa*), representing a food crop for man, and plantain (*Plantago major*), a common species of natural ecosystems. To limit the variation in wax composition between different plant "batches", due to changing environmental conditions, plants were grown under controlled conditions. To study the influence of leaf age on the wax composition, wax extracts from leaves of different developmental stages were analysed. Leaf cuticular wax of *P. major* is known to contain polar, free triterpenes (Hegnauer 1969), but its composition has never been presented in detail. For *L. sativa*, to the best of our knowledge, no data on the composition of the cuticular wax have been published in literature before.

Experimental

Plants

Lettuce (*Lactuva sativa* L., cv. Meikoningin, commercial seeds) and plantain (*Plantago major* L., wild origin) were grown under controlled conditions in water culture (Hoagland). A 12 hr

light period (21°C) was followed by a 12 hr dark period (15°C). Relative humidity was kept at 70%. $\cdot m^{-2} \cdot s^{-1}$ at plant level.

Extraction procedures

Fully expanded leaves of old (*ca*. 3 months) plantain and lettuce were weighed (typical sample wt. 5 g) and extracted by immersing them in 50 ml of chloroform, n-hexane, toluene, chloroform/methanol (2:1 v/v) or dichloromethane for 15 min. at room temperature, while shaking gently by a horizontal shaker. Extracts were evaporated to dryness under a gentle stream of nitrogen. To study the influence of leaf developmental stage on wax composition, leaves of older lettuce and plantain plants were divided into three groups: young (not fully expanded), middle age (just fully expanded) and older leaves.

To the wax extracts, 2.5 ml of 80% acetone was added. The concentration of chlorophyll was spectrophotometrically determined, according to MacKinney (MacKinney 1941).

Gas chromatography and identification

Wax extracts were analyzed by GC-FID before and after methylation with diazomethane on a 25 m CP-SIL 5CB-column (ID 0.32 mm, film thickness 0.12 µm, carrier gas N_2 at 6 psi inlet pressure). Injector and detector temp. 280°C. Oven temp. 250°C (45 min.), quickly raised to 300°C and kept at that temperature for 45 min. Peaks were identified by relative retention time compared to the internal standard 5α-cholestane. Additional identification of the wax compounds was done after separation of the leaf wax on a silica column with a stepwise elution with a diethylether-petrol gradient. Elution with 100% petrol gave the alkanes, whereas the esters were present in the 5% diethylether fraction in petrol. The alcohols were eluted by 25% diethylether in petrol and 100% diethylether eluted the triterpene acids.

Results and discussion

Wax composition

The total amount of extracted wax of *L. sativa* changed considerably with developmental stage: the young, middle age and old leaves containing 5, 11 and 8 mg wax$\cdot g^{-1}$ dry weight (DW), respectively. In contrast, its composition is little affected by leaf developmental stage (Table 1). However, only in the wax of old leaves traces of the triterpenoid precursor squalene could be detected. The wax components of fully expanded, older lettuce leaves are listed in Table 2. It mainly consists of linear primary alcohols and, to a lesser extent, of fatty acids, their biochemical precursors. In the old leaves, 9% of the components could not be identified.

Table 1. Composition (%) of chloroform extracts of leaves from *Lactuca sativa* from three developmental stages.

component	*young*	*medium*	*old*
linear alcohols	88	86	83
fatty acids	8	6	7
squalene			1
unidentified	4	8	9

Table 2. Components from methylated wax of old leaves of *Lactuca sativa*, extracted with chloroform.

component	*RT*	*% total area*	*component*	*RT*	*% total area*
henicosanol	3.92	< 1	pentacosanol	8.49	2
docosanol	5.07	26	squalene	9.04	< 1
m.e. docosanoate	5.35	< 1	hexacosanol	10.39	24
tricosanol	7.04	< 1	m.e. hexacosanoate	11.16	2
tetracosanol	7.5	22	heptacosanol	13.54	3
m.e. tetracosanoate	8.49	3	octacosanol	16.07	5
			unidentified		9

RT = retention time in minutes; m.e. = methyl ester

For *P. major*, almost no influence of leaf developmental stage on the total amount of extracted wax was found; the young leaves contained 10 mg wax·g^{-1} DW, while middle age and old leaves had 8 mg wax·g^{-1} DW. Again, the composition of the leaf wax was relatively constant with leaf developmental stage (Table 3). Table 4 summarizes the identified components from the methylated extract of cuticular wax of old leaves of *P. major*. Main compounds are the polar, non-volatile, free triterpene acids, oleanolic acid and ursolic acid, which were detected by GC-FID as their volatile methylester derivatives. Besides these triterpene acids, linear alkanes were present as major compounds. The constant composition of the extractable cuticular wax with leaf age makes the plants highly suitable as test plants in research on foliar uptake of semivolatile organic contaminants.

Table 3. Composition (%) of chloroform extracts of leaves from *Plantago major* from three developmental stages.

component	*young*	*medium*	*old*
triterpenic acids	68	64	57
linear hydrocarbons	18	13	19
linear alcohols	1	1	1
unidentified	13	22	23

Table 4. Components from methylated wax of old leaves of *Plantago major*, extracted with

chloroform.

component	*RT*	*% total area*	*component*	*RT*	*% total area*
hexadecanol	2.57	< 1	triacontane	13.52	< 1
m.e. heptadecanoic acid	2.95	< 1	henitriacontane	16.87	10
m.e. nonadecanoic acid	3.32	< 1	dotriacontane	21.20	< 1
heptacosane	7.40	< 1	tritriacontane	26.78	3
hexacosanol	10.50	< 1	m.e. oleanolic acid	35.51	14
nonacosane	10.93	3	m.e. ursolic acid	39.49	44
			unidentified		23

RT = retention time in minutes; m.e. = methyl ester

Solvents

Leaf extracts from both species made with chloroform, dichloromethane, toluene or n-hexane were colourless. However, the extracts obtained from a mixture of chloroform and methanol were green. Spectrophotometric measurements confirmed the presence of 5 µg chlorophyll per g fresh weight (FW) in the chloroform/methanol extracts of *P. major*. For the other solvents, this was less than 0.1 $\mu g.g^{-1}$ FW (extraction time 15 min.). In contrast to this, Bewick and coworkers (Bewick *et al.* 1993), already detected several $\mu g.g^{-1}$ FW of chlorophyll in the extracts of torpedograss and black nightshade after 2 s of dipping the leaves in chloroform. Apparently, the cuticles of these plants are penetrated by chloroform more rapidly compared with those of *P. major* and *L. sativa*.

Besides chlorophyll, internal cytoplasmic lipids (*e.g.* for *L. sativa*: the membrane sterols, ß-sitosterol, campesterol and stigmasterol and for *P. major*: tocopherol) were present in the extracts made with the chloroform-methanol mixture. The presence of internal compounds indicates that, in contrast to the other solvents used, this solvent penetrates into the interior of the leaf. This is due to methanol, which penetrates the cytoplasmic compartment relatively fast. For this reason, methanol or a combination of methanol with other solvents is not suitable for the extraction of leaf cuticular waxes of lettuce and plantain.

In Figure 1 the composition of extracts of *L. sativa* leaves, extracted with the various solvents, is shown. The wax compounds are grouped as linear alcohols, fatty acids, squalene and membrane sterols. The extract made with chloroform-methanol is the only one containing internal lipids. The distribution of the wax compounds over the groups is very similar for the extracts made with chloroform, dichloromethane, toluene and n-hexane (Figure 1). This indicates that these solvents extract leaf wax of *L. sativa* equally well. However, this was not the case for the leaves of *P. major*, as is shown in figure 2. In this figure the relative extracted amounts of the two dominant types of compounds of leaf wax of *P. major* (Figure 2), namely linear alkanes and triterpene acids (the sum of which is arbitrarily set to 100%), are plotted for the various solvents. The apolar solvent, n-hexane, extracts less triterpene acids relative to the linear alkanes than the other, more polar solvents.

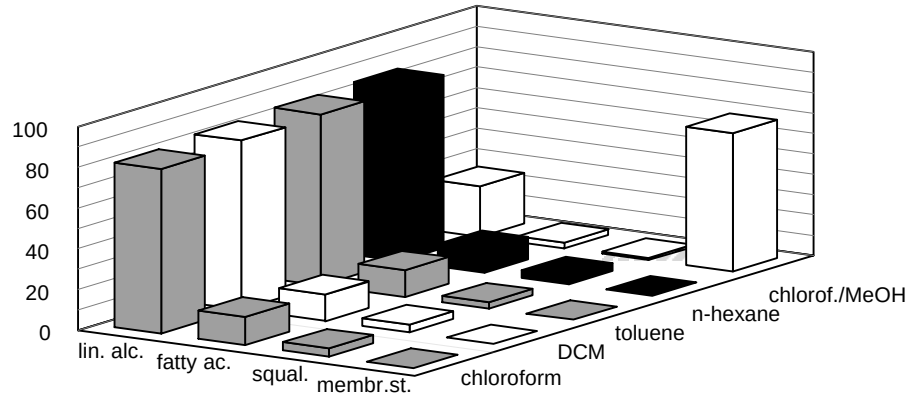

Figure 1. Composition (%) of leaf wax extracts of *L. sativa* made with chloroform, dichloromethane (DCM), toluene, n-hexane and a 2:1 (v/v) mixture of chloroform and methanol (chlorof./MeOH). Wax components are grouped as linear alcohols, fatty acids, squalene and membrane sterols. The sum of wax components is set to 100%.

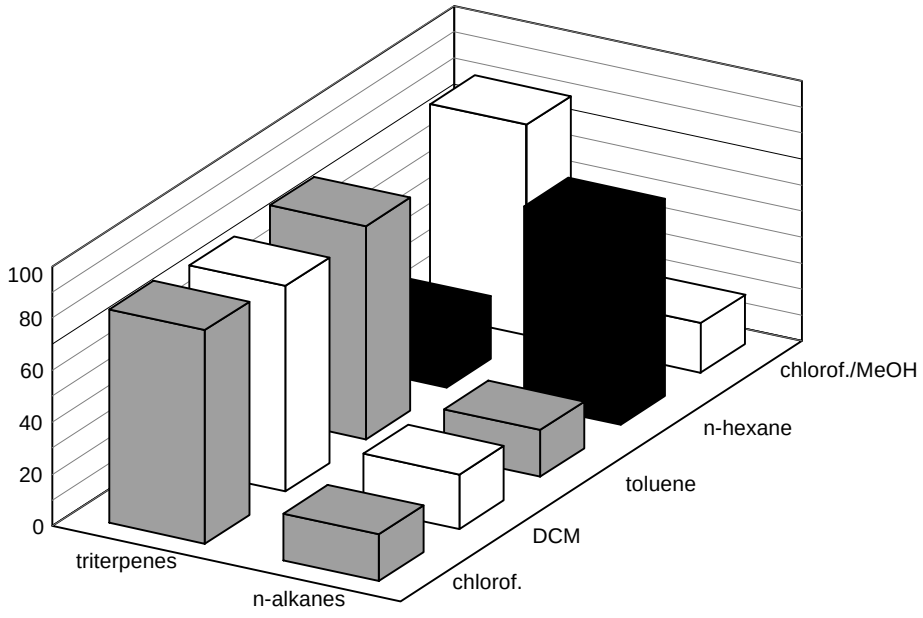

Figure 2. Relative amounts (%) of triterpenes and n-alkanes in extracts of *P. major*, made with chloroform, dichloromethane (DCM), toluene, n-hexane and a 2:1 (v/v) mixture of chloroform and methanol (chlorof./MeOH). The sum of extracted triterpenes and n-alkanes is arbitrarily set to 100%.

This finding is supported by an additional experiment, in which already extracted leaves were *post*-extracted with chloroform for 15 min (Figure 3). Whereas n-hexane extracts the linear alkanes relatively well (only 20% of the total is present in the *post*-extract) the amount of triterpene acids

present in the *post*-extract is 50 times higher as in the initial n-hexane-extract. In contrast, the *post*-extracts of two other solvents (toluene and chloroform) only contained a small amount of triterpene acids. Thus, the apolar solvent n-hexane is not suitable for extraction of the relatively polar leaf wax of *P. major*.

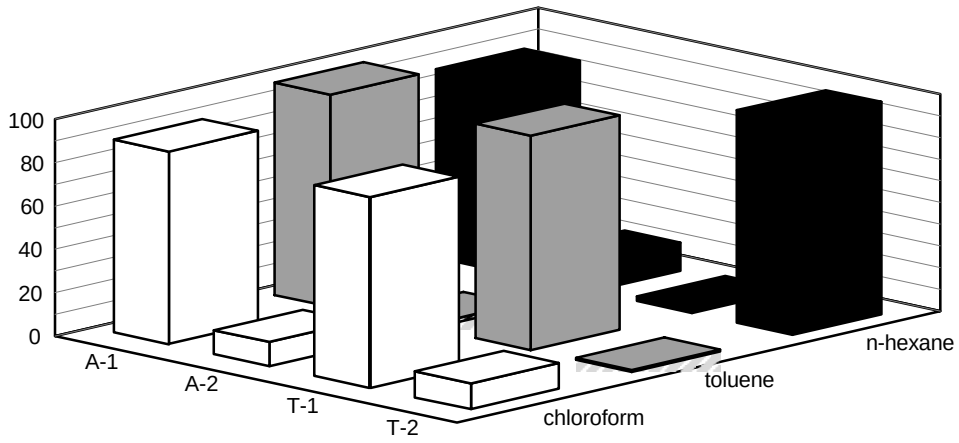

Figure 3. Relative amounts (%) of n-alkanes (A) and triterpenes (T) in main- (1) and post- (2) extracts of P. major, made with chloroform (extraction time 3 min.), toluene (extraction time 60 min.) and n-hexane (extraction time 60 min.). Post-extraction time was 15 min. The sum of main and post-extract is set to 100%.

As a consequence, although n-hexane is widely used for extracting SOCs from plant leaves, this solvent thus cannot be used for the extraction of leaf wax of intact *P. major* leaves. The presence of polar triterpene acids may inhibit the penetration of the apolar solvent into the deeper embedded cuticular wax layers and, therefore, prevent the extraction of SOCs that have diffused into these layers. However, the three more polar solvents (chloroform, toluene and dichloromethane) can be used for the extraction of SOC from cuticular wax of *P. major*.

Since we are unaware of experiments in which authors checked the efficiency of n-hexane as an extraction solvent for SOCs, we recommend that the extraction efficiency of a solvent other than chloroform (or the comparable dichloromethane) is tested for each plant species beforehand.

Acknowledgement

Many thanks to Ben Maas, Desirée van den Bergh and Ilco Brussé for carrying out the experimental work.

4

Dry deposition of ATMOSPHERIC polycyclic aromatic hydrocarbons IN THREE *Plantago* species

Adapted from:

Martine Bakker, Michel Vorenhout and Chris Kollöffel, 1999. *Environmental Toxicology and Chemistry* **18**: 2289-2294.

Abstract

The concentrations of polycyclic aromatic hydrocarbons (PAHs) in the leaf wax of three *Plantago* species were determined weekly for three weeks. The almost glabrous, free-standing leaves of *Plantago major* and the sparsely hairy *Plantago lanceolata* leaves, were more heavily contaminated with low molecular weight (MW) PAHs (MW < 228) than the densely hairy, partly overlapping *Plantago media* leaves. This may be caused by the lower canopy roughness (higher aerodynamic resistance), the higher amount of leaf hairs (higher boundary resistance) and/or the higher leaf overlap (smaller accessible leaf area) of *P. media*. On the other hand, PAHs with MW ≥ 252 tended to show higher concentrations in *P. media* than in the other two species. This is likely caused by the dense layer of hairs on *P. media* leaves, which can efficiently intercept the largely particle-bound high MW PAHs. When the PAH concentrations were normalised to projected leaf surface area the differences between *P. media* and the other two species became significant ($P < 0.05$) for the high MW PAHs, while the differences for the low MW PAHs decreased. Although the differences in PAH concentrations between species are relatively small (factor 2-5), this study clearly shows that plant architecture and leaf hairs influence the dry deposition of PAHs.

Introduction

The uptake of compounds from the atmosphere in plant leaves involves three steps (see **Chapter 2**): the transportation from the (turbulent) atmosphere to the laminar air boundary layer surrounding the leaf (1), the crossing of the boundary layer (2) and the interaction of the molecule with the leaf surface (3). The dominant resistance to the uptake of SOCs is in the atmosphere (step 1 and 2) or in the plant (step 3), depending on environmental conditions, plant characteristics and the properties of the compound.

The main compound property determining the dominating resistance is the K_{oa} (the partition coefficient between octanol and air). For compounds with a low K_{oa}, the cuticle is relatively impermeable and the plant resistance in the main resistance. In this case, the concentration differences between plants may be explained by the amount (Simonich and Hites 1994b) and composition (Kömp and McLachlan 1997a) of the lipids. On the other hand, compounds with a high K_{oa} (*e.g.* PAHs, PCDD/Fs) are highly soluble in the cuticle and therefore, atmospheric resistance limits uptake (McLachlan *et al.* 1995). The atmospheric resistance, (consisting of the aerodynamic and the boundary resistance, corresponding to respectively step 1 and 2) is influenced by the plant architecture as well as the shape of the leaf surface. The surface roughness of the canopy influences aerodynamic transport (high roughness increases transport), while the roughness of the leaf surface is one of the factors that determines the thickness of the laminar boundary layer ($\delta_{b.l}$). The $\delta_{b.l}$ is also influenced by wind speed and irradiation.

PAHs are present in the atmosphere both in the gaseous phase and bound to particles. While PAHs with MW < 252 are predominantly present in the gaseous phase, large particle-bound fractions are found for PAHs with higher MW, due to their low vapour pressures and high K_{oa} values (Jones *et al.* 1992, Kaupp 1996). The deposition of particles is dependent on particle size, plant characteristics and environmental conditions (Chamberlain and Little 1981). Several plant characteristics have been related to particle deposition. As for gases, a high aerodynamic surface roughness leads to efficient turbulent transport of particles (Burkhardt *et al.* 1995). Particle deposition on different types of vegetation was found to increase with increasing leaf area index (Jonas and Heinemann 1985, Heil 1988). Wind tunnel experiments with radio-labelled particles with sizes ranging from 0.03–44 µm have shown that hairy leaves are better particle collectors than glabrous leaves (*e.g.* Romney *et al.* 1963, Chamberlain 1967, Wedding *et al.* 1975, Little and Wiffen 1977).

Plant architecture and leaf hairs may thus affect the deposition (rate) of gases and of compounds bound to particles. In this chapter, we compare the dry deposition of gaseous and particle-bound PAHs in three species of *Plantago*, which differ in the amount of leaf hairs and in the architecture of the plant, but have similar wax characteristics.

Experimental

Chemicals

Phenantrene (PHE, MW 178), anthracene (ANT, MW 178), benz[a]anthracene (BaA, MW 228), chrysene (CRY, MW 228), and benzo[a]pyrene (BaP, MW 252) were obtained from Sigma (St. Louis, MO, USA). Benzo[k]fluoranthene (BkF, MW 252) was obtained from Chem Service (West Chester, PA, USA) and benzo[g,h,i]perylene (BghiP, MW 276) from Fluka (Buchs, Switzerland). Fluoranthene, (FLUO, MW 202) and 5α-cholestane were purchased from Aldrich (Steinheim, Germany).

Chloroform (p.a.) and diethylether (p.a.) were obtained from Merck (Darmstadt, Germany), methanol (HPLC gradient grade) and acetonitrile (ACN, HPLC gradient grade) from Baker (Deventer, The Netherlands). Octadecylsilica (C_{18}) was purchased from Baker and prewashed with methanol and ACN before use.

Plants

Plantago species are herbs with a short stem. The leaves usually arise from the base of the stem and are spirally arranged. The leaves of *Plantago major* L. (great plantain) and *Plantago lanceolata* L. (ribwort plantain) are relatively free-standing in the air, whereas the leaves of *Plantago media* L. (hoary plantain) are closely spreading on the ground and partly cover each other. *P. major*-leaves (length 10-15 cm) are broad and almost glabrous, while *P. lanceolata* has lanceolate leaves (length 10-15 cm), having more hairs, whereas *P. media* has ovate leaves (length 5-10 cm) with a dense layer of silky hairs.

P. major (seeds from wild origin), *P. lanceolata* (seeds from wild origin) and *P. media* (seeds from Meise, Belgium) were grown in a greenhouse (temp. 28°C). After 4 weeks they were put in separate pots and 5 weeks later they were transferred to a colder greenhouse (temp. 15°C). Fifteen weeks after sowing, the plants were fully grown and placed in an “open” greenhouse (missing the lower half of the walls) in the Botanical Gardens of Utrecht University. This site is considered an urban area. The distance to the nearest highway is approximately 400 m, and the distance to downtown Utrecht is approximately 4 km. The potted plants were placed close to each other, to minimize environmental variation. The plants were sprayed with collected rainwater twice a day. The temperature during the day was 29 ± 5 °C and during the night 8 ± 3

Samples

Leaf samples (8 g, quadruplicates) were taken on the last day in the greenhouse (Day 0) and on Day 6, 13 and 20. Each sample consisted of 1 leaf (*P. major*) or 3 to 4 leaves (*P. lanceolata* and *P. media*), originating from the same amount of individual plants. Leaf fresh wt. was determined, after which leaf wax was extracted and analyzed for PAHs. Finally leaf dry wt. was determined. Using plants which were not subjected to PAH analysis, the wax content, the specific leaf area (SLA, cm^2 leaf area$\cdot g^{-1}$ dry wt) and the ratio of the projected surface area (A_{proj}) to total surface area (TSA) of the three species were determined ($f_{proj} = A_{proj}/TSA$). Wax content was determined on Day 13 and Day 20 (n=1), while SLA was measured on each sampling day (n = 4). The f_{proj} were determined for a different batch of plants (n=3).

Leaf wax and leaf area

To determine the amount and composition of the leaf wax, leaves were immersed in 2×25 ml chloroform, for respectively 30 s and 10 s, to extract leaf wax. Previous experiments have shown that in this time period the leaf wax is completely extracted (data not shown). Extracted leaves were dried in an oven (80°C, 24 h) to determine foliar dry weight. Extracts were filtered with a glass microfiber filter (pore diameter 2.7 µm, Whatman, Clifton, NJ, USA) to remove particles and after evaporation to dryness, wax weight was determined. The composition of the leaf wax (redissolved in diethylether) was determined as described in **Chapter 3**.

Leaf areas were measured with a leaf area meter (Licor Lincoln L1-3100). To determine f_{proj}, the projected surface area (A_{proj}) was divided by the total surface area of the leaves (= 2x measured leaf area). A_{proj} was determined by taking photographs of the plants from a vertical view, clipping out the projected plant and measuring the area of the clippings (correcting for the scale of the photos).

Sample clean-up and PAH analysis

Leaves were not washed before extraction, to make sure that particle-bound PAHs were not washed away. Extracts (made as described in the previous section) were evaporated to 10 mL under a gentle stream of nitrogen. An amount of 1.5 g C_{18} was added and the samples were further evaporated to dryness. Clean-up of the samples was performed by transferring the C_{18} to a 20 ml plastic syringe filled with a plug of quartzwool and 1 g C_{18}, followed by elution with 18 mL of ACN. Samples were evaporated to 0.5 mL under nitrogen, and injected into an HPLC-system with a Merck-Hitachi L-6200 Intelligent Pump (Merck, Darmstadt, Germany), which

was supplied with a Chrompack ChromSpher PAH-column (Chrompack, Middelburg, The Netherlands) connected to a Merck-Hitachi F-1050 Fluorescence Spectrometer (Merck, Darmstadt, Germany). The column was eluted with ACN/millipore water 55/45 with a linear gradient to 65/35 in 6 min. A ratio of 65/35 was used for 2 min, followed by a 6 min linear gradient to 100% ACN. Then, 100% ACN was flushed for 7 min, after which the ACN/millipore water ratio was reset instantaneously to 55/45. Used wavelengths were 255 nm (excitation)/ 405 nm (emission) for all compounds, except for fluoranthene, which was measured with 280 nm/450 nm.

Quantification

The PAHs were identified by comparing the retention times of the sample peaks to those of the standards. Signal collection and data processing was performed on a computer with Chromcard 1.17 (Fisons Instruments, Loughborough, UK). Recoveries of the PAHs were > 90%, except for BghiP, which was 77 ± 9%. Procedural blanks (n = 4) were determined by extraction and cleanup with 50 ml chloroform. PAH concentrations in the samples were calculated by correcting the measured values for recovery and subtracting the average amount measured in the blanks.

Results and discussion

Cuticular waxes

The main components of the extractable cuticular waxes of *P. major* are linear alkanes ($C_{27}H_{56}$-$C_{33}H_{68}$) and triterpene acids (oleanolic and ursolic acid), (**Chapter 3**). The other two plants have a similar wax composition (data not shown). The wax contents of the plants approximately doubled from Day 13 to Day 20. Nevertheless, the wax content relative to *P. major* remained fairly constant and differed not much from 1 (for *P. lanceolata* the data were 0.82 and 0.86, for *P.media* 1.12 and 1.31 for Day 13 and Day 20 respectively). The SLA of the plants was also similar and did not show a trend with time (144 ± 5, 123 ± 5 and 165 ± 12 $cm^2.g^{-1}$ dry wt for *P. major*, *P. lanceolata* and *P. media* respectively). However, f_{proj} of *P.media* was significantly ($P<0.01$) less than that of the other two species (0.22 ± 0.07, in contrast with *P. major* and 0.48 ± 0.08 for *P. lanceolata*).

Time course of PAH concentrations and PAH-profiles

PAHs were already present in the wax of the leaves from the greenhouse (Day 0). The PAH concentrations in the plants (Figure 1) increased by a factor of 2-7 after they were transferred to the open greenhouse (with the exception of BghiP). This increase was likely caused by a higher supply of PAHs or smaller boundary layers around the leaves due to more wind in the open greenhouse. Concentrations on Day 6 and 13 were similar, but on Day 20 concentrations of most compounds decreased considerably (Figure1). The reason for this decrease remains unclear. The only change from Day 13 and Day 20 in the measured plant parameters was the twofold increase of the wax content, but this cannot explain a decrease in PAH-concentration expressed per g of leaf dry weight. Nevertheless, for the purposes of this study, namely the interpretation of differences between species, it is not of much relevance.

The profiles of the PAHs of P. major and P. lanceolata were very similar, while the profiles of P. media showed a slightly different pattern. This is mainly due to a consistently lower contribution of PHE (40-55% instead of 50-60%) and a higher contribution of higher MW PAHs to the total amount of PAHs taken up (data not shown). For example, BkF accounts for ± 4% in P. media and for ± 1% in the other two plants. FLUO also made up a major proportion of the total concentration (25-40%). The rest of the PAHs were only present in small amounts (0.5-10%).

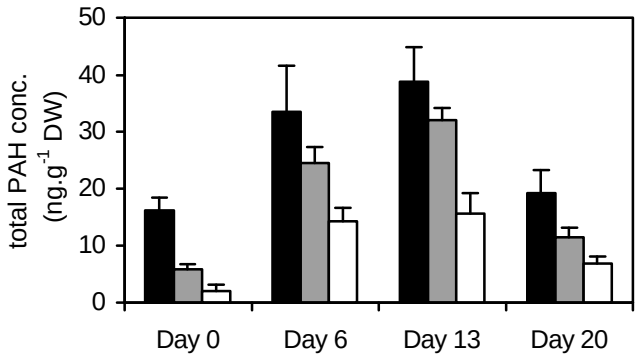

Figure 1. Total PAH concentrations (ng PAH. g^{-1} dry wt. of leaf) of eight PAHs in leaf wax of *P. major* (black bars), *P. lanceolata* (hatched bars) and *P. media* (white bars) on the different sampling days. Error bars represent standard deviations of the four replicates.

Species differences

To compare the concentrations of PAHs in the three Plantago species, the results of Day 13 are plotted in Figure 2. On the other sampling days, the species differences were similar, and therefore the results of these days are not shown. Concentrations are expressed per g dry weight, since this parameter was measured for all samples, in contrast to the leaf area and the

wax content, which were only determined for a number of control plants. However, the differences in the wax content and in the SLA between the species are relatively small and plots based on these units result in similar figures, showing the same significant differences as Figure 2.

The concentrations of PAHs with MW 178 and 202, which are largely present in the atmosphere as gases (Jones *et al.* 1992, Kaupp 1996), were 2-5 times higher in *P. major* and *P. lanceolata* than in *P. media* (Figure 2).

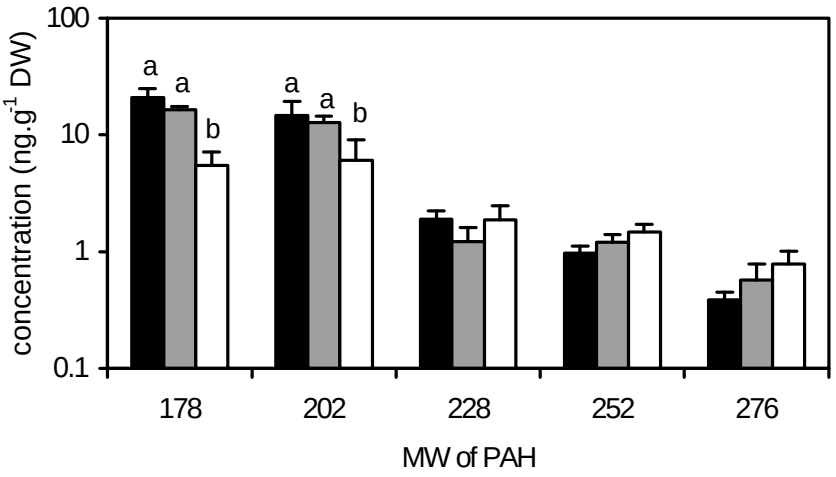

Figure 2. PAH concentrations (ng PAH·g^{-1} dry wt of leaf) in leaf wax of the three *Plantago* species on Day 13. PAHs are grouped according to their molecular weight (MW). Differences between the average concentrations of the three species were tested with ANOVA (Prism 2.01, P<0.05) and when found significant, indicated in the figure with letters a and b.

For the higher MW PAHs, which are almost completely bound to particles (Jones *et al.* 1992, Nakajima *et al.* 1995, Kaupp 1996), significant differences between the species could not be proved statistically (Figure 2). The absence of statistical significance could be caused by the limited sample size that could be cleaned up efficiently. Hence, the amount of high MW PAHs that was extracted is relatively low (down to approximately 1 ng) resulting in low precision. Nevertheless, when looking at the trends, concentrations of PAHs with MW 252 and 276 in *P. media* are highest and those in *P. major* lowest. This was also the case for the individual PAHs with MW 252, namely BaP and BkF. The same trend was found on the other sampling days as well.

The differences in PAH concentrations can be explained by the differences in plant characteristics of the three *Plantago* species. These can be divided in differences in surface roughness, leaf hairs and leaf overlap.

Surface roughness

Because *P. media* is a low growing plant with its leaves spreading close to the ground, the aerodynamic surface roughness of the canopy will be lower than that of the other two plants. This will result in less aerodynamic turbulence and therefore, to a lower supply of compounds from the bulk air. However, this will only lead to lower uptake if the aerodynamic component of the atmospheric resistance is the rate-limiting step.

For particle-bound PAHs it is expected that the lower surface roughness of *P. media* will have a similar effect on the deposition. Since the pattern of high MW PAHs in Figure 2 points in the opposite direction, the surface roughness cannot explain this finding and is probably over-compensated for by another factor, such as leaf hairs and leaf overlap.

Leaf hairs

Surface irregularities on the leaf, such as veins and hairs can induce turbulence and hence, decrease $\delta_{b.l.}$. On the other hand, a dense mat of hairs is likely to increase $\delta_{b.l.}$ by up to the thickness of the hair mat (Jones 1983). The effect of hairs on $\delta_{b.l.}$ was demonstrated by Woolley (Woolley 1965), who showed that the wind speed 0.5 mm above a soybean leaf increased by 40% after the hairs had been removed. In contrast with the other two plants, *P. media* leaves are densely hairy and therefore the $\delta_{b.l}$ will be increased. This may cause a lower uptake rate for the gaseous PAHs, if the boundary resistance is the main atmospheric resistance.

It is not clear from this experiment whether the turbulent or the laminar component of the resistance determined gas transport to the leaf surface. Therefore, no conlusions can be drawn about the actual cause of the lower uptake of the low MW PAHs in *P. media*. Both the lower surface roughness and the higher density of leaf hairs of *P. media* are possible explanations for this phenomenon.

The trend of *P. media* having the highest concentrations of high MW PAHs (Figure 2), can be explained by its hairy leaves. PAHs are largely bound to particles < 2 µm (Poster *et al.* 1995, Schnelle *et al.* 1995, Allen *et al.* 1996, Kaupp 1996). It is known that in the size range 1-5 µm, the deposition by impaction (inertial motion) is inefficient and the presence of fine hairs may be of major importance in intercepting particles (Chamberlain 1967). For particles smaller than 1 µm, diffusion becomes the dominant means of transport to the leaf surface, and the nature of the surface is not so important as for larger particles. However, once the particles have been deposited on the leaf surface, the hairy leaves act as an efficient particle trap. Because of the thick boundary layer, wind eddies cannot penetrate down to blow off the particles from the leaf surface (Gregory 1961). Another explanation for the increased particle collection efficiency of hairy leaves is that leaf hairs may cushion the impact and therefore reduce the bounce-off of particles (Chamberlain and Little 1981).

Leaf overlap

The lower concentrations of gaseous PAHs in *P. media* may also be caused by the higher overlap of leaves of this plant. Because of the overlap, the leaves may be less accessible for exchange of air, thus preventing the uptake of gaseous SOCs in the covered leaves. This factor is not taken into account when expressing PAH concentrations per g dry wt, per g leaf wax or per m^2 surface area. Therefore, we normalised the PAH concentrations (Day 13) on the projected surface area, A_{proj}, which could be calculated for each sample from the measured total surface area and f_{proj}, which was determined for the control plants.

PAH concentrations based on A_{proj} are plotted in Figure 3. Concentrations of PAHs with MW 178 were again lowest in *P. media*, while the concentrations of FLUO (MW 202) were similar in the three plants. This suggests that A_{proj} may at least partially explain the observed differences for the gaseous compounds. After normalisation to A_{proj}, the high MW PAHs showed significantly higher concentrations in *P. media* than in the other two plants (Figure 3). This means that per unit "accessible" surface area, the concentrations of particle-bound PAHs in *P. media* are significantly higher than those of the other two plants.

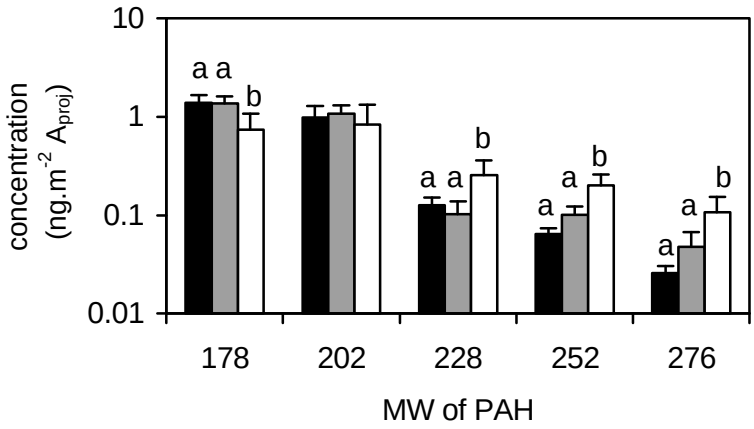

Figure 3. PAH concentrations (ng PAH·m^{-2} projected area) in the leaf wax of the three *Plantago* species on Day 13. PAHs are grouped according to their molecular weight (MW). Error bars represent standard deviations of the four replicates (taking into account the error in the determination of A_{proj}). Differences between the average concentrations of the three species were tested with ANOVA (Prism 2.01, P<0.05) and when found significant, indicated in the figure with letters a and b.

The projected surface area (A_{proj}) represents the surface area that is not covered by other leaves. Since in this approximation the vertical dimension is lost, the use of A_{proj} is only acceptable when using plants with highly similar morphology. Although the plants used in the present study belong to the same genus, they differ in height, which complicates the interpretation. The greater height of *P. major* and *P. lanceolata* may increase the interception of

PAHs. However, in spite of the possibly extra deposition caused by their height, high MW PAH concentrations in *P. major* and *P. lanceolata* expressed per projected area (A_{proj}) were lower than those of *P. media,* which emphasizes the effective particle collection of hairy leaves.

Since A_{proj} probably reflects a "minimal accessible leaf area", only a rough idea of the influence of the plant architecture on the deposition of PAHs can be obtained in this way. Besides, since gases are able to diffuse much faster than particles, due to their higher diffusion coefficients, the use of A_{proj} as a measure of accessiblity will likely be of more relevance for particle-bound compounds than for gaseous compounds.

Kinetics

The explanations given in the previous sections consider the atmospheric resistance. The atmospheric resistance can only determine the measured concentrations if an equilibrium between air and plant has not been reached. This is because in an equilibrium situation, the concentrations in the plant are independent of the aerodynamic turbulence and boundary layer thickness. Because of the lack of data for the kinetic behaviour of PAHs in *Plantago*, the time needed to reach equilibrium was estimated from data measured for other plants. Since the elimination rate decreases with increasing K_{oa} (Paterson *et al.* 1991), only studies in which compounds were used with log K_{oa} values comparable to those of PHE, ANT and FLUO (which have a log K_{oa} of respectively 7.4 -Tolls and McLachlan 1994-, 7.8 -Tolls and McLachlan 1994- and 8.6, calculated from De Maagd *et al.* 1998), were chosen.

From the elimination rate constants reported in these studies, the time needed to achieve 95% of the equilibrium concentration ($t_{0.95}$) was calculated with the first-order one-compartment kinetic bioconcentration model. In studies in which plants were exposed in chambers containing a ventilator, i.e. under turbulent conditions (thin boundary layers), $t_{0.95}$ values ranged from 3 days for anthracene in grass (Tolls and McLachlan 1994) to 107 days for PCB#18 in spruce needles (Reischl *et al.* 1989). Under non-turbulent lab conditions a value of 31 days for mirex (Bacci *et al.* 1990b) was found, whereas in a field study concentrations of chlorinated organic compounds (PCBs, DDT *et al.*) in spruce needles were still increasing after 5 years (Jensen *et al.* 1992). These results indicate that it is not likely that equilibrium will be reached within three weeks of exposure in an "open" greenhouse.

Conclusion

The densely hairy, partially overlapping leaves of the low growing *P. media* contain less low MW PAHs and more high MW PAHs than the almost glabrous, *P. major* and sparsely hairy *P. lanceolata* leaves, which are relatively free-standing in the air. The measured trends are consistent and indicate that leaf hairs and plant architecture can affect deposition rates of SOCs in different ways for gases and particles.

These results may be important for predictive models, in particular with regard to the concentration of SOCs in food crops. Food crops are only exposed to contaminated air for a relatively short time period and an equilibrium between air and plant will probably not be achieved. Neglecting the effects of plant architecture may result in overestimation of concentrations of gaseous SOCs in the plant. On the other hand, deposition of particle-bound SOCs may be underestimated for leaves with a dense layer of hairs. However, differences in foliar concentrations of SOCs between plant species in these experiments were not large; usually less than a factor of 5.

5

LOCALISATION of deposited PAHs in leaves of *Plantago*

Adapted from:
Martine Bakker, Judith Koerselman, Johannes Tolls and Chris Kollöffel, *Environmental Toxicology and Chemistry*, accepted for publication May 2000.

Abstract

After deposition to foliage, atmospheric PAHS may remain on the leaf surface, accumulate in the cuticular wax or diffuse into the remaining "interior" of the plant. In a field study, the location of deposited PAHs in the leaves of two *Plantago* species was determined. To this aim, leaves of *P. major* and *P. media* were divided into three fractions. Firstly, the leaves were washed (wash-off fraction), then cuticular wax was extracted (wax fraction). Finally, the remaining leaf material was extracted (interior fraction). The presence of PAHs could be demonstrated in all three fractions. For both plants, the distribution of PAHs over the three fractions changed with molecular weight (MW) of the PAHs. The wash-off fraction increased with increasing MW, likely because high MW PAHs occur predominantly bound to particles, which can be readily washed off from the leaves. In contrast, the amount of PAHs detected in the interior of the leaves decreased with increasing MW. This can be explained by a slow desorption of the PAHs from the particles and a low diffusion rate of the larger molecules. This study shows that washing reduces the amount of high MW PAHs on plant surfaces. Therefore, washing of leafy vegetables is important to minimize human dietary intake of PAHs.

Introduction

During accumulation of SOCs in plants, compounds may first adsorb to the surface and then diffuse into the cuticle of the plant (Schreiber and Schönherr 1992). In several studies in which needles and leaves were separated in a cuticular wax and an inner compartment by means of fractionated extractions, SOCs were present in both compartments (Reischl *et al.* 1987, Hauk *et al.* 1994, Umlauf *et al.* 1994b, Kaupp 1996, Wenzel *et al.* 1998). Hence, compounds may accumulate in the cuticular waxes, but may also diffuse through the waxes to accumulate in the cutin or possibly the interior of the needle/leaf.

PAHs are deposited to plant surfaces by gaseous and particle-bound deposition (Nakajima *et al.* 1995, Kaupp 1996). The question arises whether the fate of deposited particle-bound PAHs is analogous to that of gaseous PAHs. Larsson and Sahlberg (Larsson and Sahlberg 1982) demonstrated that washing of lettuce with water removed a considerable amount of the high MW PAHs, but little of the small PAH phenantrene. Therefore, they suggested that only PAHs with a low molecular weight (MW) could be sorbed into the cuticle. High MW PAHs, mainly associated with particles, would remain on the leaf surface (Larsson and Sahlberg 1982). In contrast with these findings, Kaupp (1996) rinsed corn leaves with water and EDTA solutions and found that only a small fraction of the high MW PAHs and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) was removed in this manner. This suggests that compounds can be desorbed from particles and diffuse into the cuticle or that the particles may be encapsulated in the cuticle (Kaupp 1996).

Because of the conflicting results of the studies mentioned above, the location of deposited PAHs in or on plant leaves is unclear. The aim of the present study was to localise PAHs in leaves of *Plantago major* and *Plantago media*. To this end, we determined the distribution of PAHs over three fractions of plant leaves: (1) “attached” to the leaf surface, (2) sorbed in the extractable cuticular wax and (3) in the interior of the leaf.

Experimental

Chemicals

The PAHs were obtained as described in **Chapter 4**. In addition pyrene (PYR) was purchased from Sigma (St. Louis, MO, USA) and benzo[e]acephenantrylene (BeA, used as internal standard) was bought from Aldrich (Steinheim, Germany). EDTA was obtained from Aldrich as well. Serdolit (PAD I) was purchased from Brunschwig (Amsterdam, The Netherlands) and was prewashed with acetonitrile (ACN), methanol and ethanol before use. Methanol (HPLC gradient

grade) and ACN (HPLC gradient grade) were obtained from Baker (Deventer, The Netherlands). Dichloromethane (DCM, HPLC grade) was purchased from Merck (MSD, Haarlem, The Netherlands). All other solvents were of analytical quality and obtained from Baker (Deventer, The Netherlands).

Plant and site description

P. major L. (with broad, almost glabrous and relatively free-standing leaves) and *P. media* L. (ovate leaves with a dense layer of silky hairs) were grown in a greenhouse (temp. 28°C). In July 99, at the age of 5 months, the plants were planted in a plot at the Botanical Gardens of Utrecht University. To prevent contact of the leaves with the soil, the ground was covered by a plastic cloth. Small pieces were cut from the cloth, after which the plants were placed in the created holes. The soil under the holes was covered with small pebbles. During the experiment, temperatures varied from 13 to 23 °C (average 18.6 °C). The experiment lasted 72 days, with rain events on 19 days (6 days > 5 mm rain). From Day 57 until 72 the plants were watered twice a week (without wetting the leaves), since in this period there was only 1 mm of rainfall (data from weather station De Bilt, which is 1.4 km from the site). After 72 days in the field, the plants were harvested. From *P. media* plants young leaves (3-5 weeks old) were sampled from the top and old leaves (8-10 weeks) from the lower parts of the plant. From *P. major*, only young leaves (5-7 weeks) were sampled.

Samples

Leaf samples (15 g, quadruplicates) consisted of 3 to 4 leaves (*P. major*) or 5 to 10 leaves (*P. media*), originating from 2 individual plants. Leaf fresh weight was determined. The samples were separated into three fractions. Firstly, they were washed by shaking with 2 * 100 ml (for 90 s and 30 s, respectively) of an EDTA solution (3×10^{-2} M, pH 5) in a beaker. The EDTA solution was extracted by refluxing with 100 ml cyclohexane for 10 min. Following the washing procedure with EDTA, leaves were immersed in 2×45 ml DCM, for respectively 30 s and 10 s, to extract cuticular wax. After the DCM was concentrated until ± 1 ml under a nitrogen flow, extraction was performed according to the method of Kaandorp *et al.* (1990). First, 45 ml of methanolic KOH was added. Subsequently, the solution was refluxed for 30 minutes. Cyclohexane (100 ml) was added and this was refluxed for 10 minutes. After extraction of cuticular wax, the leaves were ground with liquid nitrogen, 45 ml of methanolic KOH were added and the same procedure was followed as used for the cuticular wax. All cyclohexane fractions (washing water, cuticular wax and leaf interior) were concentrated to 1 ml under a

gentle stream of nitrogen. Five ml of MeOH was added and the solution was concentrated to 2 ml. Clean up of the samples took place on columns (∅ 10 mm) filled with 2.2 g Serdolit. After addition of the sample, EtOH (5 ml), pentane (5 ml) and EtOH (5 ml) were eluted and discarded. ACN (45 ml) and EtOH were eluted. This was evaporated to 0.5 mL under nitrogen and analyzed by HPLC with fluorescence detection, as described in **Chapter 4**. Recoveries of the PAHs varied from 72 to 90 %, except for BkF and BaP, which amounted to 64 ± 14 and 47 ± 12 %, respectively. Procedural blanks were determined by extraction and cleanup of 90 ml DCM (n = 2) or 45 ml MeOH/KOH (n = 3).

Control samples

Procedural blanks were determined by extraction and cleanup of 90 ml DCM (n = 2) or 45 ml MeOH/KOH (n = 3). Recovery of the PAHs was determined by spiking a known amount of PAHs to leaf samples with known background concentrations, and calculating the percentage recovered after cleanup (subtracting the background). Recoveries of the PAHs varied from 72 to 90 (± 10-13) %, except for BkF and BaP, which amounted to 64 ± 14 and 47 ± 12 %, respectively.

Using plants which were not subjected to PAH analysis, the leaf dry weight, the wax content and the specific leaf area (SLA, cm^2 leaf area·g^{-1} dry wt) of 15 g leaf material (n=1) were determined according to the methods described in **Chapter 4**.

The efficiency of the washing procedure with EDTA solution was checked and compared with that of water. This was done by washing leaves three times with 100 ml water or EDTA solution instead of two times with EDTA solution (n = 3).

Quantification of PAHs

BghiP (MW 276) could not be determined quantitatively, due to the presence of an interfering peak of an unknown substance in the chromatogram. The presence of phenantrene in the fractions was sometimes difficult to prove, due to high concentrations of PHE in the procedural blanks (72 ± 18 ng). Quantification of BaA and CRY also suffered from relatively large blanks (7 ± 3 and 6 ± 1 ng, respectively). Only values that exceeded the average concentration in the blanks plus two times the standard deviations were taken into account.

PAH concentrations were calculated by correcting for recovery percentages and subtracting the average amount measured in the blanks. Reproducibility of the measurements was relatively low: the relative standard deviation varied from 5 to 80%, being < 50% in 60% of the cases. PAH concentrations are expressed on a dry weight basis. The measured leaf

parameters (fresh weight, leaf surface and wax content) are similar for the different groups of leaves. Therefore, plots based on these parameters will result in similar figures.

Results and discussion

Evaluation of the fractionation procedure

In the control experiment, no differences were found between the washing efficiency of water and the EDTA solution. Although EDTA was mentioned as effective for removing particles from leaves (Kaupp 1996), in this study water appears just as efficient.

Washing leaves for a third time with 100 ml (instead of twice) rendered solutions without amounts of PAHs with MW of 178, 202 or 228 exceeding those in the blanks. This demonstrates that these compounds were already washed off by the first 200 ml. However, significant amounts of BkF and BaP (MW 252) were present in the third washing solution. The behaviour of BkF and BaP was similar. Whereas 69 ± 2% and 16 ± 3% were present in the first and second washing solution, respectively, in the third wash 13 ± 2% of the two compounds was found.

The effect of cross-contamination of the other fractions is likely smaller, but cannot be completely ruled out. Although the wash-off fractions did not contain measurable amounts of cuticular wax, small quantities of wax may have eroded during the washing procedure, thereby contributing to the amount of PAHs in the wash-off fraction. Extraction of PAHs from the interior of the leaves during cuticular wax extraction is not very likely, as during the short extraction times (30 + 10 s) chlorophyll was not extracted (**Chapter 3**). Vice versa, no large contribution of wax-borne PAHs is expected in the interior fraction, as experiments have shown that additional cuticular wax was not extracted with DCM after 30 s (data not shown). However, it should be noted that the collected fractions are operationally and not physically defined.

PAH concentrations of whole leaves

The PAH concentrations of the whole leaves were calculated by adding the amounts of the three fractions, and dividing the total amount by the dry weight (DW) of the sample. The PAH concentrations of the whole leaves (PAHs grouped by MW) varied from several tens of nanograms to more than 1 µg per g dry weight (Figure 1). In this study, MW 178 represents PHE and ANT; MW 202 is FLUO and PYR. MW 228 is BaA and CRY, while MW 252 represents BkF and BaP.

The differences between the PAH concentrations of the two kinds of *P. media* leaves (Figure 1) are caused by the differences in exposure period: old *P. media* leaves (8-10 weeks) have higher concentrations than young ones (3-5 weeks).

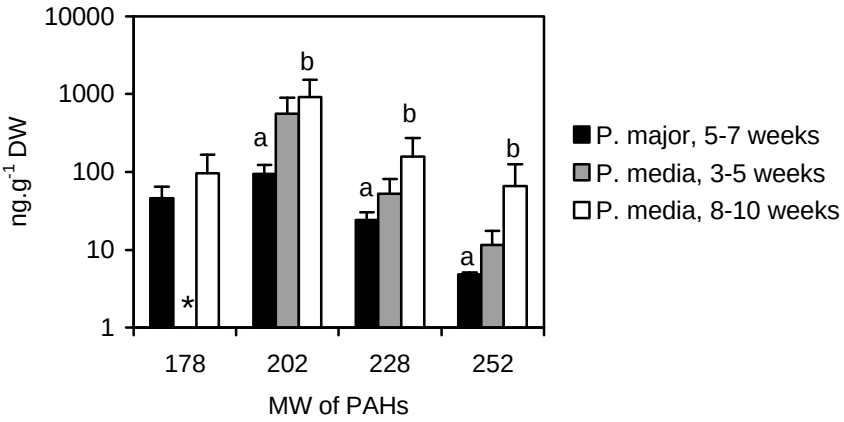

Figure 1. PAH-concentrations (ng·g^{-1} DW) in the leaves of *P. major* (5-7 weeks old, black bars) and in young leaves (3-5 weeks, hatched bars) and old leaves (8-10 weeks, white bars) of *P. media.* Error bars represent standard deviations of the four replicates. Statistically significant differences between the average concentrations of the groups (ANOVA, Prism 2.01, P < 0.05) are indicated in the figure with letters a and b. An asterisk marks the case in which the amount of PAHs in the samples was less than the amount in the procedural blanks plus two times the standard deviation (for MW 178 *i.e.* < 108 ng).

While young *P. media* leaves have the shortest exposure period, the PAH concentrations of MW 202, 228 and 252 in these leaves are higher than those in *P. major* (5-7 weeks). The only exception is formed by PAHs with MW 178, which presence could not be shown in young leaves of *P. media.* As high MW PAHs are predominantly bound to particles in the atmosphere (Jones *et al.* 1992, Kaupp 1996), the higher concentrations of these compounds in young *P. media* leaves are probably due to their hairy surface (see **Chapter 4)**.

As the hairy leaves of *P. media* can more effectively retain particle-bound PAHs than the glabrous *P. major* leaves (**Chapter 4**), it was expected that the wash-off fraction of high MW PAHs was higher for *P. media* than for *P. major*. Although the average wash-off fraction of the old *P. media* leaves was indeed consistently higher than that of *P. major*, the differences were not statistically significant. This is due to the relative high variation in the data.

Distribution of PAHs over three fractions

The distribution of the PAHs over the three fractions - wash-off, cuticular wax and leaf interior- changes with the MW of the PAHs for both plant species (Figure 2). The fraction found in the washing solution increases with increasing MW of the PAHs, while the fraction in the leaf interior decreases. This trend is evident for all three kinds of leaves and will be discussed below.

The PAH distribution over the three fractions is similar for the three groups of leaves. Although the wash-off fractions seems to be largest for old *P. media* leaves and smallest for

leaves of *P. major*, the differences are not statistically significant due to the relatively large standard deviations of the PAH concentrations of the fractions.

Wash-off fraction

The increase of the wash-off fraction with increasing MW of the PAHs is most likely due to the wash-off of particles. As high MW PAHs have larger particle-bound fractions in the atmosphere, this leads to a higher contribution of particle-bound deposition to plant leaves. This is in agreement with McLachlan's framework for the interpretation of measurements of SOCs in plants (McLachlan 1999). In this framework, the dominant deposition processes are distinguished as a function of the compound's K_{oa}. For compounds with high K_{oa} values, such as high MW PAHs, particle-bound deposition is the controlling process.

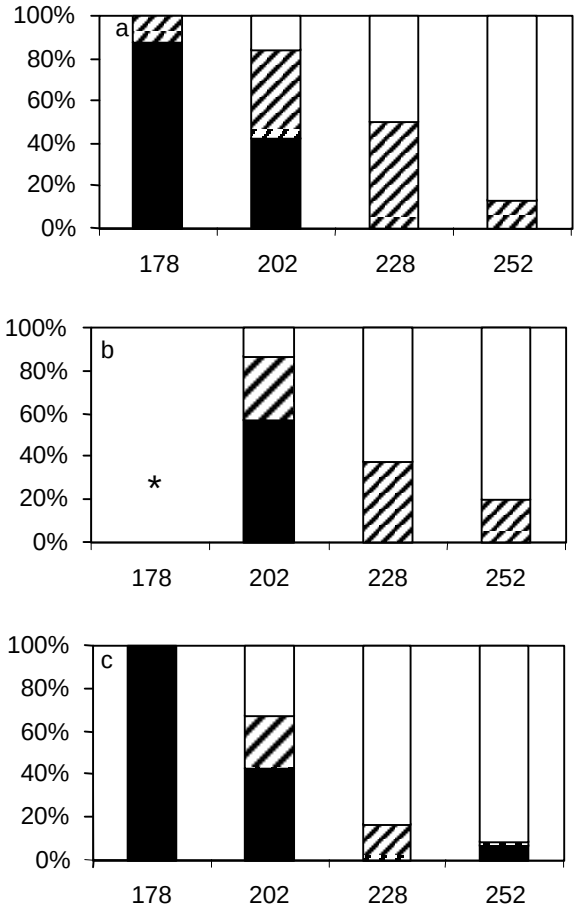

Figure 2. Distribution of PAHs over three fractions: wash-off (white bars), extractable cuticular wax (hatched bars) and leaf interior (black bars) for (a) leaves (5-7 weeks old) of *P. major*, (b) young leaves (3-5 weeks) of *P. media* and (c) old leaves (8-10 weeks) of *P. media*. An asterisk marks the case in which none of the fractions did contain amounts exceeding those in the procedural blanks plus two times the standard deviation (for MW 178 *i.e.* 108 ng).

Due to the inefficiency of the method used to wash off PAHs with MW 252, as mentioned earlier, the wash-off fraction of this group of compounds is somewhat (10-15%) underestimated in Figure 2. Consequently, the fraction in the cuticular wax is overestimated, due to the amount that was not washed off with EDTA, but extracted together with the cuticular wax. Correction of the fractions for this artefact makes the observed trend (increasing wash-off fraction with increasing MW of the compounds) even more evident.

The presence of the high MW PAHs in the wash-off fraction agrees well with the results of the study of Larsson and Sahlberg (1982). These authors also showed a large wash-off of high MW PAHs: washed lettuce leaves contained only a part of the PAHs of non-washed leaves (30–60% for PAHs with MW 228, 13-17% for MW ≥ 252). In contrast, concentrations of the low MW compound PHE in washed leaves amounted to 90% of the concentrations of the non-washed leaves. However, the results of Kaupp's study (1996) are different. This author found 4% of high MW PAHs and PCDD/Fs in water which was used to rinse corn leaves, and at most 20% in the EDTA solution in which the leaves were subsequently shaken. Together, only 24% of the high MW compounds were located at the leaf surface. It is not clear what causes the different results of Kaupp (1996) on the one hand and those of Larsson and Sahlberg (1982) and the present results on the other hand. The differences may be due to differences in plant characteristics, the thoroughness of the washing procedure and the environmental conditions. For example in this study the collection of paticles was probably very effective, since there was hardly any "natural washing" during the experiment (less than 1 mm of rain during the last three weeks). This may have resulted in relatively high amounts of particle-bound PAHs. On the other hand, the relatively high temperatures during the experiment may have increased the gaseous to particle-bound concentration ratio of PAHs in the atmosphere. In addition, differences in roughness of the leaf surface (particle collection efficiency) and/or in epicuticular wax characteristics (particle embedding efficiency) between the plants of the different studies may have been responsible.

Interior fraction

In contrast with the wash-off fraction, the fraction of PAHs that is present in the interior of the leaves decreases with increasing MW of the compound (Figure 2). This can be explained by the fact that low MW PAHs can reach the leaf interior in a relatively short time. High MW compounds will need more time to cross the cuticular waxes. As compounds can move through the cuticle exclusively by molecular diffusion, their mobility is inversely proportional to their molar volume (Schreiber and Schönherr 1993, Schreiber 1995). Furthermore, as these compounds occur predominantly bound to particles, they will have to desorb from the particles before entering the cuticle. As these compounds are very hydrophobic, they have a low tendency to desorb from the particle and therefore entering the wax. Another entry route may be the encapsulation of the PAH-particle complex by the cuticular wax. As the complex will have a

lower diffusion rate due to its larger volume, this process will take considerably more time than diffusion of a molecule of a low MW PAH.

Whereas PAHs with MW 178 and 202 are present in the interior of all leaves (with one exception, see Figure 2), only the interior of old *P. media* leaves contained PAHs with MW 252, in this case BkF (Figure 2). The other leaves probably did not live long enough to allow diffusion of BkF into their interiors. This demonstrates that a compound with MW 252 can traverse the cuticular wax of these leaves within a period of 8–10 weeks. The presence of PAHs with MW 228 in the leaf interiors could not be demonstrated, due to the relatively high blanks of these compounds.

The occurence of high MW compounds in the interior of needles and leaves is consistent with other research (Reischl *et al.* 1987, Hauk *et al.* 1994, Umlauf *et al.* 1994b, Kaupp 1996, Wenzel *et al.* 1998). In several of these investigations (Reischl *et al.* 1987, Umlauf *et al.* 1994b, Kaupp 1996), just as in the present study, the amount of compound found in the inner leaf (or needle) was correlated to the MW of the compound. In contrast with the findings of this study, large fractions of high MW compounds are sometimes found in the interior of pine needles (Reischl *et al.* 1987, Wenzel *et al.* 1998). This can be explained by the long lifetime (up to several years) of pine needles compared to that of leaves.

Conclusion

The location of airborne PAHs in leaves of *P. major* and *P. media* is related to the molecular weight of the compounds. Low MW PAHs, for which gaseous deposition is dominating, are predominantly present in the cuticular wax and the interior of the leaves, whereas high MW PAHs, deposited via particles, mainly remain at the leaf surface. Washing of the leaves with either water or EDTA solutions efficiently reduces the amount of high MW PAHs, including the potent carcinogens benzo[k]fluoranthene and benzo[a]pyrene. Consequently, although the plant species and the weather conditions in this study may not be representative for the growing of food crops in general, this shows that human intake of high MW PAHs can be reduced by the thorough washing of vegetables.

Acknowledgement

Manuela Haller and Lucy Huibers are acknowledged for carrying out cleanup and introductory experiments, respectively.

6

Aerodynamic SURFACE ROUGHNESS affects deposition Of atmospheric PAHs TO *PLANTAGO MEDIA*

Adapted from:

Martine Bakker, Judith Koerselman, Johannes Tolls and Chris Kollöffel, manuscript submitted as a short communication to *Environmental Toxicology and Chemistry*, May 2000.

Abstract

Atmospheric polycyclic aromatic hydrocarbons (PAHs) can be deposited to plants. The deposition of PAHs was studied in a field experiment using *Plantago media*, a herbaceous species with leaves spreading closely to the ground and partly covering each other. The architecture of the plants was varied by cutting leaves from the top of the plants. On the one hand, the old leaves of intact plants are more overlapping than the old leaves of clipped plants, which may result in a lower accessible leaf area. On the other hand, intact plants have a larger total leaf area per plant (estimate of leaf area index), and hence, a higher aerodynamic surface roughness than clipped plants. As leaves of intact plants contained more PAHs than leaves of clipped plants (on leaf area basis: a factor of ~6), this result indicates that the aerodynamic surface roughness of plants is more important for atmospheric deposition of PAHs than leaf overlap.

Introduction

Plants can accumulate airborne semivolatile organic compounds (SOCs), such as polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and polychlorodibenzo-p-dioxins and -furans (PCDD/Fs), (*e.g.* Simonich and Hites 1995a). This accumulation process causes concern, as it causes indirect human exposure to semivolatile organic compounds; for PAHs mainly via the consumption of leafy vegetables (Edwards 1983) and for PCBs and PCDD/Fs predominantly via the consumption of meat and dairy (*e.g.* McLachlan 1996).

SOCs are deposited to plants by three different deposition mechanisms, one of each dominating at a different range of the octanol-air partition coefficients (K_{oa}) of the compounds (McLachlan 1999). With increasing K_{oa}-values, the dominant deposition mechanisms are (1) equilibrium partitioning of gases, (2) kinetically limited gaseous deposition and (3) particle-bound deposition (McLachlan 1999). Each deposition mechanism is influenced by specific plant characteristics.

In equilibrium partitioning - the dominant deposition mechanism for compounds with a relatively low K_{oa} - the content and composition of plant lipids are the main factors determining the accumulation (Simonich and Hites 1995a, Kömp and McLachlan 1997a, Böhme *et al.* 1999). For the other two deposition mechanisms, -prevailing for SOCs with a higher K_{oa} - equilibrium between air and plant is not approached. For these compounds, the rate limiting step in the uptake process is in the atmosphere (McLachlan 1999). Therefore, the uptake is limited by the rate at which the compound can be transported to the plant (McLachlan 1999).

As the transport from the atmosphere to the laminar boundary layer around the leaf occurs by turbulent wind eddies, and the surface shape is important in influencing air flow patterns around the plant, dry deposition is strongly determined by the aerodynamic surface roughness of the plant (*e.g.* Davidson and Wu 1990). The aerodynamic surface roughness is determined by the plant architecture, particularly by the total surface area per unit ground surface (leaf area index), (Jonas and Heinemann 1985, Heil 1988) and/or the herbage density (total surface area per volume of air), (Chamberlain 1970, Schuepp 1989). Also, the number and size of the vegatitive elements as a function of height has been used to estimate aerodynamic surface roughness (Davidson *et al.* 1982).

For particle-bound deposition, other factors besides the aerodynamic surface roughness are also essential, such as the roughness of the leaf surface, *e.g.* the presence of leaf hairs (**Chapter 4**) and the orientation of the leaves (Welsch-Pausch and McLachlan 1996, Böhme *et al.* 1999). Although the influence of the aerodynamic surface roughness and the leaf orientation is discussed in several publications (*e.g.* Monteith 1973, Heil 1988, Welsch-Pausch and McLachlan 1996, Böhme *et al.* 1999), the role of plant architecture in atmospheric deposition of SOCs is not very well known. In a previous paper, differences in PAH-concentrations between

three *Plantago* species were attributed to differences in aerodynamic surface roughness (aerodynamic resistance), amount of leaf hairs (boundary resistance) and/ or leaf overlap (accessible leaf area), (**Chapter 4**). To get more specific information on the effect of plant architecture on SOC deposition, we investigated the effect of clipping leaves from the top of *Plantago media* plants, leaving a ring of older leaves at the plant base. By the removal of leaves the effect of leaf overlap and aerodynamic surface roughness could be studied. *P. media* was chosen as a test plant, as this plant has a relatively high leaf overlap. This investigation was undertaken together with a field study described elsewhere (**Chapter 5**).

Materials and methods

Plantago media L. (hoary plantain) is a herbaceous plant with a flat rosette of leaves. The leaves closely spread on the ground and partly cover each other. The plants were grown in a greenhouse and at the age of five months placed on a field in the Botanical Gardens in Utrecht (an urban area). One group of leaves, described in the previous study as “old” leaves , was collected from the ring of oldest leaves of intact *P. media* plants (**Chapter 5**). The second group of leaves was collected from ten individual *P. media* plants, which were grown in the greenhouse and transferred to the field at the same time as the intact plants. The two groups of plants were placed in the field in a mixed configuration. Five weeks after the transfer to the field, the leaves of the second group of plants were removed, except for those from the oldest “ring” of leaves. This resulted in “clipped” plants, with leaves spreading low to the ground and little leaf overlap. New, young leaves were removed twice a week.

After the harvest (plants of both groups at the same time, ten weeks after the transfer to the field), the concentrations of the following eight PAHs were determined: phenantrene (PHE), anthracene (ANT), fluoranthene (FLUO), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CRY), benzo[k]fluoranthene (BkF) and benzo[a]pyrene (BaP). The methods used for extraction, cleanup and analysis are described in the previous paper (**Chapter 5**).

Leaves from both groups of plants were about 8-10 weeks old. The fresh weight/dry weight (FW/DW) ratio and the wax content were measured (as described in **Chapter 4**) for two individual plants from each group which were not subjected to PAH analysis. Leaf areas of these plants (total leaf area-one side of the leaves- and the projected leaf area –leaf area of the whole rosette-) were determined with a leaf area meter (Licor Lincoln L1-3100). Statistical tests were performed with a computer programme (t-test, Prism 2.01).

Results and discussion

The architecture of the plants was affected by removing the upper leaves from *P. media*. The clipped plants differed from the intact ones in the height of the plant (~1.5 cm vs. ~4 cm), the number of leaves (5-6 vs. 15-20), the total leaf area (310 ± 70 cm^2 vs 470 ±110 cm^2) and the ratio total leaf area/ projected leaf area (1.05 ± 0.1 vs. 1.52 ± 0.2).

The concentrations per m^2 leaf area for the PAHs (grouped by their molecular weight MW) were higher for the leaves of the intact plants than for those of the clipped plants, on average a factor of 5.6 (Figure 1). In this Figure, MW 178 represents PHE and ANT, MW 202 is FLUO and PYR, MW 228 represents BaA and CRY, while MW 252 is BkF and BaP. The sum of the eight PAHs is also shown. The differences between the two plants are statistically significant for the sum of the PAHs and for PAHs with MW 202. For the other MWs, the difference is not statistically different, due to a relatively high variation in the data. When the concentrations are normalized to wax content or leaf dry weight, the resulting plots are similar, although the concentration differences are larger (factor ~11 and ~ 9, respectively), yielding statistically significant differences for PAHs with MW 202 and 228 (data not shown).

In the following, the difference between the PAH concentrations will be discussed with respect to the dominant deposition mechanism, which changes with a changing K_{oa} of the compound.

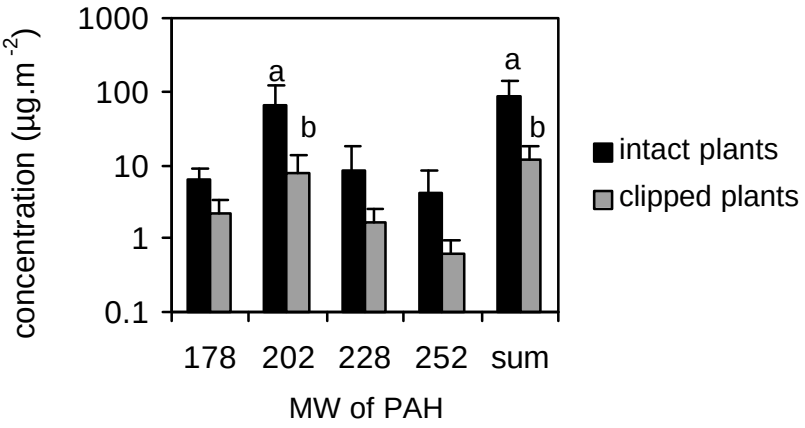

Figure 1. PAH-concentrations (µg·m^{-2}) in 8-10 week-old leaves of intact plants (black bars) and of clipped plants (hatched bars) of *P. media.* PAHs are categorized in groups according to their molecular weight (MW). Total concentrations (sum of 8 PAHs) are also given. Error bars represent standard deviations of the four replicates.

For low MW PAHs, equilibrium between the plant and the atmosphere may have been reached (McLachlan 1999). In a field study in Germany, it has indeed been found that phenantrene and anthracene (MW 178) reached equilibrium in a part of the tested plant species (Böhme *et al.* 1999). Under equilibrium conditions, the variation in the concentrations in the

different plant species is reduced when expressing the concentrations on a lipid basis. However, in this study the differences between the plants are larger when the concentrations are normalized on the wax content (factor 11 instead of 6). Although the extractable cuticular wax is only a rough estimate of the total amount of plant lipids (Böhme *et al.* 1999), we consider it not likely that the different amounts of total lipids in the two groups of plants are responsible for a concentration difference of a factor of 11. Therefore, in this study, equilibrium has probably not yet been reached for these compounds, and kinetically limited gaseous deposition is most likely the controlling deposition mechanism, just as it is for PAHs with MW 202 (Böhme *et al.* 1999). For PAHs with MW 228 and 252, particle-bound deposition will be the prevailing deposition process (Böhme *et al.* 1999).

As the age of the leaves, the presence of leaf hairs and the orientation of the leaves are all similar for the two groups of plants, the accessible leaf area and the aerodynamic surface roughness remain the significant plant characteristics in this study.

The ratio of the total leaf area/ projected leaf area of the intact plants was 1.52 ± 0.2, which means that 34 % of the leaves of intact plants were covered by other leaves, while for clipped plants this percentage was 5 % (ratio 1.05 ± 0.1). Thus, intact plants have a higher leaf overlap than clipped plants. Since the leaves of *P. media* spread close to the ground and hence, the distance between the leaves is small, the higher leaf overlap of the intact plants may result in a lower accessible leaf area. However, the leaves of the intact plants have higher PAH concentrations than those of clipped plants, so this phenomenon appears not significant.

Another effect of the higher leaf overlap of the intact plants may be that the more shielded leaves may experience less wash-off by rainfall. As a previous experiment has shown that rinsing *P. media* leaves with water reduces the amount of high MW (particle-bound) PAHs (**Chapter 5**), this may have led to higher particle-bound concentrations in the intact plants. However, during the last 16 days of the experiment, there was only 1 mm of rainfall (**Chapter 5**). Hence, the wash-off of particle-bound PAHs was probably not a significant process in this study.

The second crucial parameter, the aerodynamic surface roughness, can be estimated by the leaf area index (total surface area per unit ground surface) and/or the herbage density (total surface area per unit air volume). These factors are indeed found to correlate with the dry deposition of aerosol particles (Chamberlain 1970, Jonas and Heinemann 1985) and of nitrogen and sulphur compounds (Heil 1988, Schuepp 1989). In this study, the total leaf area per individual plant has been determined, which is a measure for the leaf area index. The total surface area per plant and thus the aerodynamic surface roughness was a factor of 1.5 higher for the intact than for the clipped plants. The higher aerodynamic surface roughness most likely caused the higher concentrations of both gaseous and particle bound PAHs of the intact plants.

Acknowledgement

We thank Roland Bobbink for his helpful comments on this chapter.

7

Uptake of gaseous SOCs in two *Plantago* **MEASURED** in a flow-through system

Abstract

Uptake of 1,2,3,4-tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene in the leaves of *Plantago major* (old leaves) and *Plantago media* (young and old leaves) was measured in a flow-through exposure system. For each specific chlorobenzene, the estimations of the uptake and elimination rate constants for the three groups of leaves were similar, despite the differences in aerodynamic surface roughness, leaf overlap and amount of leaf hairs between the leaves. This indicates that the plant side and not the air side resistance is the dominating resistance for these compounds in the system. Leaf samples were separated into an extractable wax and remaining "interior" fraction. Uptake of the compounds into the interior of leaves occurred within 3 hours. The relative amount in the interior of the leaves decreased with an increasing MW of the compound.

Introduction

Concentrations of PAHs with low MW were lower in *Plantago media* than in *Plantago major* (**Chapter 4**). The lower aerodynamic surface roughness (higher aerodynamic resistance), the densely hairy leaves (higher boundary resistance) and/or the higher overlapping of leaves (lower accessible leaf area) of *Plantago media* were held responsible for this finding. Further experiments (**Chapter 6**) showed that the aerodynamic surface roughness and not the leaf overlap appeared to be significant when the concentrations of low MW PAHs in the leaves from clipped *P. media* plants were compared to those of intact plants. So, the different concentrations in the two plants could be explained by differences in plant architecture and leaf hairs. The plant architecture can only have an effect on the concentrations in plants when the uptake is limited by the transport in the atmosphere. Therefore it can be concluded that the rate-limiting step in the deposition of PAHs (which have relatively high K_{oa} values) is in the atmosphere and not in the plant.

For SOCs with relatively low K_{oa} values (such as chlorobenzenes), the rate-limiting step of the uptake process resides in the plant (cuticle) and not in the atmosphere (McLachlan *et al.* 1995). As a consequence, the uptake rate will be dependent on the hydrophobicity of the compound. Therefore, plant characteristics as lipid composition and content are significant, instead of the plant architecture.

In this chapter the uptake of gaseous SOCs in *P. major* and *P. media* is investigated in a flow-through exposure chamber. The test compounds used in this study, namely three chlorobenzenes, two PCB and three PAHs, cover a range of K_{oa} values (from a $\log K_{oa}$ of 5.64 for 1,2,3,4-tetrachlorobenzene -Harner and Mackay 1995- to 8.88 for fluoranthene -Harner and Bidleman 1998-. For the compounds with relatively high K_{oa} values, we expect to find a similar behaviour of the compounds as the PAHs in the field experiment: higher concentrations in *P. major* than in *P. media*.

SOCs accumulate in the extractable cuticular waxes of plants, but are also found in the remaining "interior" of leaves and needles, after the waxes have been extracted (Reischl *et al.* 1987, Hauk *et al.* 1994, Umlauf *et al.* 1994b). In a previous (semi-field) experiment, PAHs were shown to be present in the leaf interiors of *P. major* and *P. media* (**Chapter 5**). The distribution over the two compartiments varied with the MW of the compound. While low MW PAHs (MW 178/202) were present in the interiors of young leaves of both plants (3-7 weeks old), benzo[k]fluoranthene (MW 252) was only present in the interiors of leaves which were 8-10 weeks exposed to ambient air. In order to get more detailed information about the time needed for a molecule to traverse the extractable waxes, leaf samples are separated in an extractable wax and an interior fraction.

Materials and methods

Exposure system

A flow-through exposure system was developed, which consisted of a 250 L acrylic exposure chamber (a glove box), through which filtered, moisturized air (± 7 $L \cdot min^{-1}$) was pulled by a pump (Figure 1). The contaminants were supplied via another, small airflow (25 $ml \cdot min^{-1}$), using a "generator column". The two air flows joined in the exposure chamber, where they were mixed by a fan. The air outlet of the exposure chamber was connected to a sampling trap.

To contaminate the air, air from a cylinder was led over 5 empore disks (Varian, Houten, The Netherlands, octadecyl silane, ∅ 47 mm) which were placed in series in filter holders in a thermostated bath (25°C). The empore disks were loaded with the contaminants 1,2,3,4-tetrachlorobenzene (TeCB), pentachlorobenzene (PeCB), hexachlorobenzene (HCB), 2,4,4'-trichlorobiphenyl (IUPAC No. 28), 2,2',5,5'-tetrachlorobiphenyl (IUPAC No. 52), phenantrene, anthracene and fluoranthene. The compounds were loaded onto the disks by dissolving an amount in a small volume of hexane. The disks were immersed in the solution and the solvent was evaporated under a small stream of nitrogen.

Exposure

Plantago major (L.) and *Plantago media* (L.) plants were placed together into the exposure chamber. Three different leaf samples were collected: one from *P. major* (± 8 g FW), and two from *P. media*, one of young and one of old leaves, respectively (± 5 g FW). Air and plant samples were taken after 3, 8, 22, 30, 46 and 54 hours.

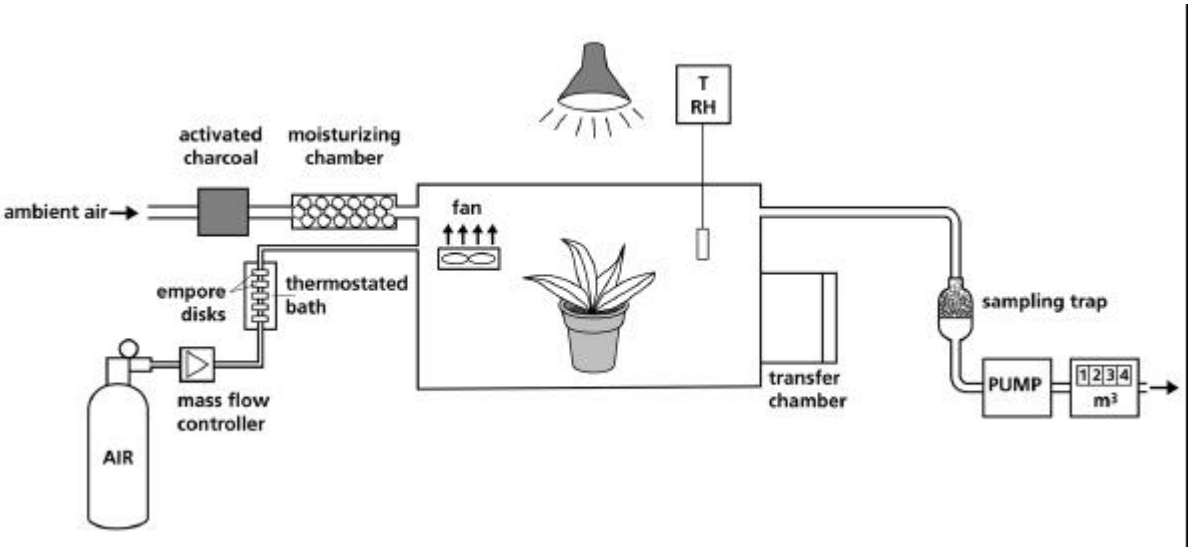

Figure 1. Flow-through exposure system for semivolatile organic compounds in plants.

Sampling and analyis

Contaminants in the air of the exposure chamber were collected by leading 100 L of air over the sampling trap (sampling time 15 minutes), containing 1 g Tenax (Chrompack, prewashed with water, aceton and hexane). They were extracted from the Tenax by shaking with hexane (18 $ml \cdot g^{-1}$). Recoveries of all compounds exceeded 87%. Procedural blanks were taken by sampling 100 L of ambient air.

Leaf samples were removed from the exposure chamber via a "transfer-chamber" (Figure 1). To extract cuticular wax, the samples were, after fresh weight determination, dipped into 2 × 45 ml DCM, for 30 s and 10 s, respectively. Subsequently, the surface area was determined by photocopying the leaves, and clipping and weighing of the copies. The dewaxed leaves were frozen in liquid nitrogen, ground in a mortar and extracted as described in a previous paper (**Chapter 5**). Cleanup of all samples was performed on activated florisil (5 g) columns, prepared from 10 ml plastic syringes. Sodium sulfate (1 ml) was added to the columns to dry the extract. Columns were eluted with 100 ml hexane, which was concentrated under nitrogen. The recovery of the compounds was > 75%, except for TeCB and PeCB, which had a recovery of 64%.

For three non-exposed samples of the two plant species the fresh weight/dry weight ratio and the wax content was determined as described in **Chapter 4**. The surface area of these samples was measured as described above. To examine the level of contamination of the leaves before exposure in the chamber, non-exposed leaves (n=2) were analysed. Procedural blanks (n=2) were determined by extraction and cleanup of pure solvents. Concentrations were calculated by correcting for recovery and subtracting the amount in the procedural blanks.

Samples (2 µl) were analysed for chlorobenzenes and PCBs with a splitless injection into a Carlo Erba (5300 series) GC equipped with ECD. Column: DB5, 30 m, i.d. 32 mm. Oven: 100°C, $\cdot min^{-1}$ to 300°C (5 min.). Measurement of PAHs was performed with an HPLC equipped with fluorescence detection as described in **Chapter 4**.

Uptake and elimination rate constants were calculated with a first order one-compartment kinetic model by a computer programme (Scientist, Micromath Scientific Software).

Results and discussion

Concentrations in air and plant samples

The concentrations of the three chlorobenzenes in the air of the exposure chamber during the experiment were 9.0 ± 4.1, 1.3 ± 0.4 and 0.2 ± 0.1 $ng \cdot L^{-1}$ for TeCB, PeCB and HCB, respectively (average and standard deviation for 8 samples). Concentrations of the PCBs and PAHs in the air could not be determined, as these were similar to the amounts in the procedural

blanks. As TeCB, PeCB and HCB were the only compounds that could be quantified in the air, we analysed these compounds in the plant samples. The variation in the air concentrations was relatively high. Therefore, all plant concentrations presented here were divided by the average air concentrations in the time prior to the leaf sampling time (*e.g.* for sampling time 22 hours, air concentrations of timepoint 0, 3, 8 and 22 hours were averaged). In the graphs shown in this chapter, concentrations are expressed per unit surface area of leaves, as this was determined for each sample and dry weight was not.

Already after 3-8 hours of exposure (the first and second leaf sampling time), the ratio of the concentration in the leaves and the concentration in the air is fairly constant for all three compounds (Figure 2). This indicates that an equilibrium between plant and air has been rapidly achieved. Therefore, calculations of uptake and elimination rate constants (first order one-compartment kinetics) are not precise: standard deviations of the constants were either extremely large or could not be calculated. Estimated uptake and elimination constants of the compounds were 10-16·10^4 L·m^{-2}·h^{-1} and 31-39 h^{-1}, respectively, in all groups of leaves. The estimated uptake rate constants increased and the elimination rates decreased with increasing K_{oa} of the compounds. However, as the standard deviations were large and the differences between the three compounds were small, the dependency of the constants on K_{oa} is not considered significant.

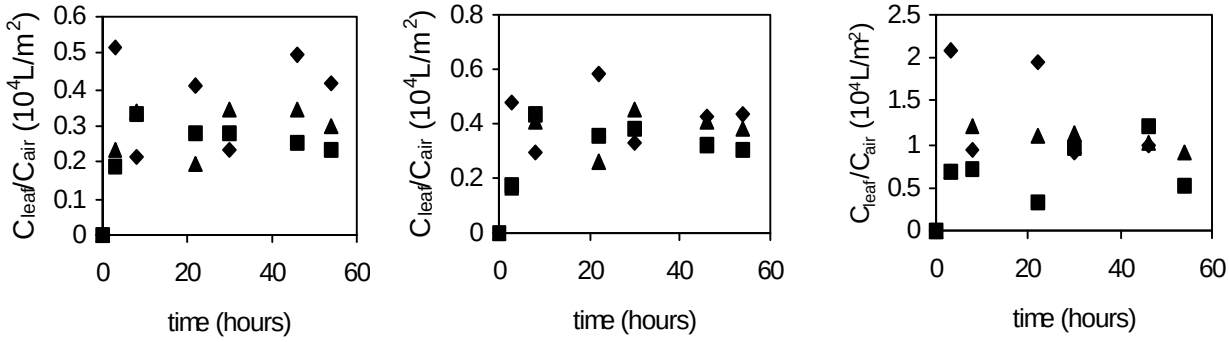

Figure 2. Leaf/air concentration ratios (10^4 L·m^{-2} leaf area) plotted versus time (hours) for TeCB (left), PeCB (middle) and HCB (right) of *P. major* (diamonds), and young (squares) and old (triangles) leaves of *P. media*.

Although the groups of leaves differ with respect to aerodynamic surface roughness, amount of leaf hairs and leaf overlap, the uptake and elimination rates were similar for the different groups of leaves. This indicates that the dominating resistance in the uptake of these compounds resides in the plant (cuticle), and not in the air surrounding the plant. If the air side resistance were dominating, the difference in plant architecture would have resulted in different uptake kinetics for the different plant species, as was the case for the PAHs in the experiments described in **Chapter 4**

and **5**. In case of plant side limitation, similar values of the uptake and elimination rate constants for all groups of leaves are not surprising, as the two plants have a similar wax composition (**Chapter 4**).

Since chlorobenzenes have relatively low K_{oa} values, their behaviour is in agreement with McLachlan's framework for deposition of SOCs to plant surfaces (McLachlan *et al.* 1999).

Chlorobenzenes in leaf wax and interior

The chlorobenzene concentrations were determined in the extractable cuticular wax and in the remaining interior of the leaves. Already at the first timepoint, after 3 hours of exposure, chlorobenzenes were detectable in the leaf interiors (Figure 3). In this figure results are shown for old leaves of *P. media*, but the other groups of leaves display a similar behaviour.

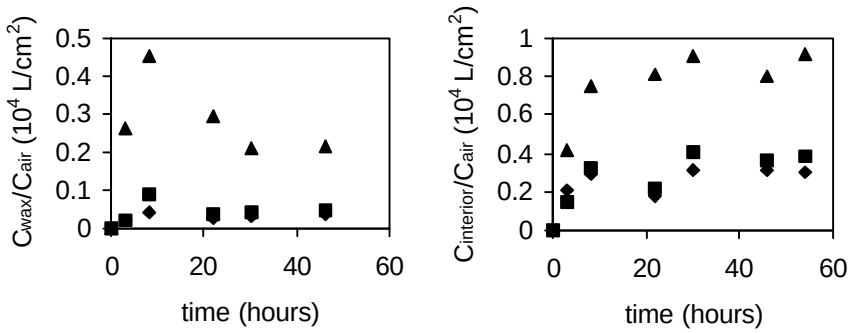

Figure 3. Concentrations in leaf wax (left) and interior (right) divided by concentration in air ($L \cdot m^{-2}$ leaf area), plotted versus time for TeCB (diamonds), PeCB (squares) and HCB (triangles) in old leaves of intact *P. media* plants.

After 20 hours of exposure, the concentrations in the leaf wax and interior are fairly constant (Figure 2), indicating that an equilibrium has been reached. On the other hand, the ratio of the concentration in the interior of the leaf and the concentration in the waxes (average value for the samples from time points > 20 h) decreases with the MW of the compound (Table 1). This finding implies that the larger compounds diffuse slower to the leaf interior than the smaller ones and that the plants were not in equilibrium.

Table 1. Ratio of concentrations in interior and wax (average of timepoints > 20 h)

leaves	*TeCB*	*PeCB*	*HCB*
P. major	3.8 ± 1.3	2.1 ± 1.1	0.7 ± 0.4
P. media, young	6.3 ± 2.0	5.5 ± 2.4	1.6 ± 1.3
P. media, old	8.5 ± 1.3	8.1 ± 2.0	3.6 ± 0.8

An explanation for this contradiction may be that the outer layers of the cuticle are in equilibrium with the air, while the deeper layers are not. In this view, the cuticle is not considered a homogeneous, but a porous medium. The compounds may transfer from the outer layers of the cuticle to deeper layers by partitioning from the wax to the air in the pores and back; the more hydrophobic compounds penetrating slower than the less hydrophobic ones (see also **Chapter 9**). During this process, the total concentration will only slowly increase, giving the "pseudo" equilibrium seen in Figure 2.

The dependence of the interior/wax concentration ratio on the MW of the compound has also been shown in earlier studies, for PAHs in *P. major* and *P. media* (**Chapter 5**), and for organic halogenated compounds and PAHs in pine needles and corn leaves (Reischl *et al.* 1987, Umlauf *et al.* 1994b, Kaupp 1996).

The concentration ratios of *P. major* are lower than those of *P. media* (Table 1). This may be explained by differences in the wax contents of the two plants. Whereas in the two previous experiments the wax contents of the two plants were similar (**Chapter 4-6**), in this experiment the amount of wax of *P. major* was higher than that of young and old leaves of *P. media* (9.3 ± 6.5 mg/g DW versus 3.1 ± 0.9 mg/g DW and 2.8 ± 0.6 mg/g DW). Although the differences are not statistically significant, all three measured wax contents of *P. major* were higher than those of *P. media*. As the higher amount of wax of *P. major* contains relatively more SOCs than the wax of the other leaves, this may have caused the lower interior/wax concentration ratio of this plant.

The bioconcentration factors of the three compounds in the two plants are similar (Figure 2), despite the higher wax content of *P. major*. This illustrates the important role of the leaf interiors, since in general they contain the main portion of the chlorobenzenes (Table 1).

Acknowledgement

Desirée van den Bergh, Bas Bokkers and Inge van de Klundert are acknowledged for their help with the development of the flow-through system. Many thanks to Manuela Haller and Judith Koerselman for the analytical work, Philipp Mayer for his advice in empore disk matters and Gert de Lange and Gijs Nobbe from the Geochemistry Department for the loan of the glovebox.

8

PAHs in soil AND PLANT SAMPLES from the vicinity OF AN OIL refinery

Adapted from:
Martine Bakker, Berta Casado, Judith Koerselman, Johannes Tolls and Chris Kollöffel, *The Science of the Total Environment*, in press.

Abstract

Soil samples, and samples of leaves of *Plantago major* and grass (mixed species) were collected from the vicinity of an oil refinery in Zelzate, Belgium, and analysed for seven polycyclic aromatic hydrocarbons (PAHs). The samples from the site adjacent to the refinery (site 1) contained very high total PAH-concentrations: namely 300, 8 and 2 $\mu g \cdot g^{-1}$ dry weight for soil, *P. major* and grass, respectively. Concentrations in samples from more remote sites (up to 4 km from the refinery) were a factor of 10-30 lower than those from site 1, but between them the differences were small. The PAH-profiles of the plant samples, in contrast with those of the soil samples, appeared to shift to higher contributions of gaseous PAHs with increasing distance from the refinery. This can be explained by particle-bound PAHs being deposited closer to the source than gaseous PAHs. It is suggested that particle-bound deposition is relatively more important for soil than for plants, due to blow-off and wash-off of the compounds from the leaves. The total PAH-concentrations in the leaves of *P. major* were higher than those measured in the grass samples, probably due to differences in aerodynamic surface roughness, leaf orientation and/or leaf age. However, the concentration ratios of *P. major*/grass were not constant for the different sites, varying from 1.2 to 8.8. Therefore, it appears that a precise prediction of PAH-concentrations for one plant species from known concentrations of another species is not possible. When errors in predicted concentrations need to be smaller than a factor of about 10, the sampling strategy has to be focussed on all species of interest.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) occur naturally in the environment, due to their formation during forest fires and vulcanic eruptions. However, the largest amount of PAHs is released into the environment by human activities (Edwards 1983). Cracking of crude petroleum, burning of fossil fuels, incineration of industrial and domestic waste, heating, cigarettes, fireplaces, etcetera, all lead to the anthropogenic formation of PAHs (Neff 1979). These compounds are semivolatile organic compounds (SOCs) and can be deposited to plant surfaces (Buckley 1982, Simonich and Hites 1995a). This accumulation process may cause indirect exposure of humans to PAHs, many of which are carcinogens or mutagens, through the consumption of fruits and vegetables.

As anthropogenic activities are the main sources of PAHs, the levels of PAHs in soils in urban areas are about a factor of 2-10 higher than those in rural areas (Lodovici *et al.* 1994, Tremolada *et al.* 1996, Wagrowski and Hites 1997, Wenzel *et al.* 1997). In addition, PAH-concentrations in soils close to highways have similar concentrations as soils in urban areas, (*e.g.* Larsson and Sahlberg 1982, Wang and Meresz 1982, Brorström-Lunden and Skärby 1984). Furthermore, PAH-concentrations in the immediate surroundings of point sources will be increased. For example, high concentrations in soils have been found close to a large scale chemical fire involving 10,000 ton of polypropylene (Meharg *et al.* 1998). In plants, higher levels have also been measured near point sources, *e.g.* the polypropylene fire mentioned above (Meharg *et al.* 1998) and an alumina smelter (Larsson and Sahlberg 1982).

One of the activities that is known to produce large amounts of PAHs is the cracking and refinery of oil. In Zelzate, a small town in Flanders (Belgium), an oil refinery plant is situated in the centre of the town. PAH-concentrations, which were measured under the authority of the Province of East-Flanders in soil and vegetable samples from gardens in Zelzate in the summer of 1997, were very high. The total concentrations of 16 PAHs were up to 200 $\mu g \cdot g^{-1}$ dry weight for soil, which exceeds Belgian and Dutch standards for PAHs. In vegetables 1-2 $\mu g \cdot g^{-1}$ dry weight was determined, which is a factor of 40 higher than reference samples from a nearby town of similar size (Provinciaal Centrum voor Milieuonderzoek 1997).

In the present study, soil and plant samples were collected in Zelzate, to investigate the influence of distance from the source on PAH-concentrations. Furthermore, the PAH-levels in different plant species were compared, in order to examine whether concentrations in different species relate to each other in a constant ratio. In risk assessment studies, such a ratio may be used as a conversion factor, to estimate concentrations in non-analysed plants species from known concentrations of analysed species. In this way, the number of samples that should be collected after an incident, and thus cost and time, may be reduced.

Experimental

Chemicals and samples

PAHs were obtained as described in **Chapter 4**. Serdolit (PAD I), methanol, and ACN were purchased as decribed in **Chapter 5**. n-Hexane (> 95%) was purchased from Baker and redistilled prior to use. All other solvents were of analytical quality and obtained from Baker.

Samples were collected in October 1997 at four different sites in the vicinity of the oil refinery of Zelzate, Belgium. Three sample locations were chosen in the north-east direction (the prevailing direction of the wind) of the oil refinery (Figure 1). The distance to the oil refinery was 50 m (site 1), 1.3 km (site 2) and 3.5 km (site 3). At each site a soil sample (soil depth 0 -1 cm) and leaf samples of *Plantago major* (n = 4, except for site 3, for which n= 2) and grass (n = 4) were taken. The fourth sample site was located south-west of the oil refinery, at a distance of about 4.2 km. At this site only grass and soil were sampled. Soil samples were collected in Erlenmeyer flasks and after transportation, stored at 10 °C. Plant samples were weighed, wrapped in aluminum foil, transported in boxes (4°C) and subsequently frozen (-20°C) until extraction.

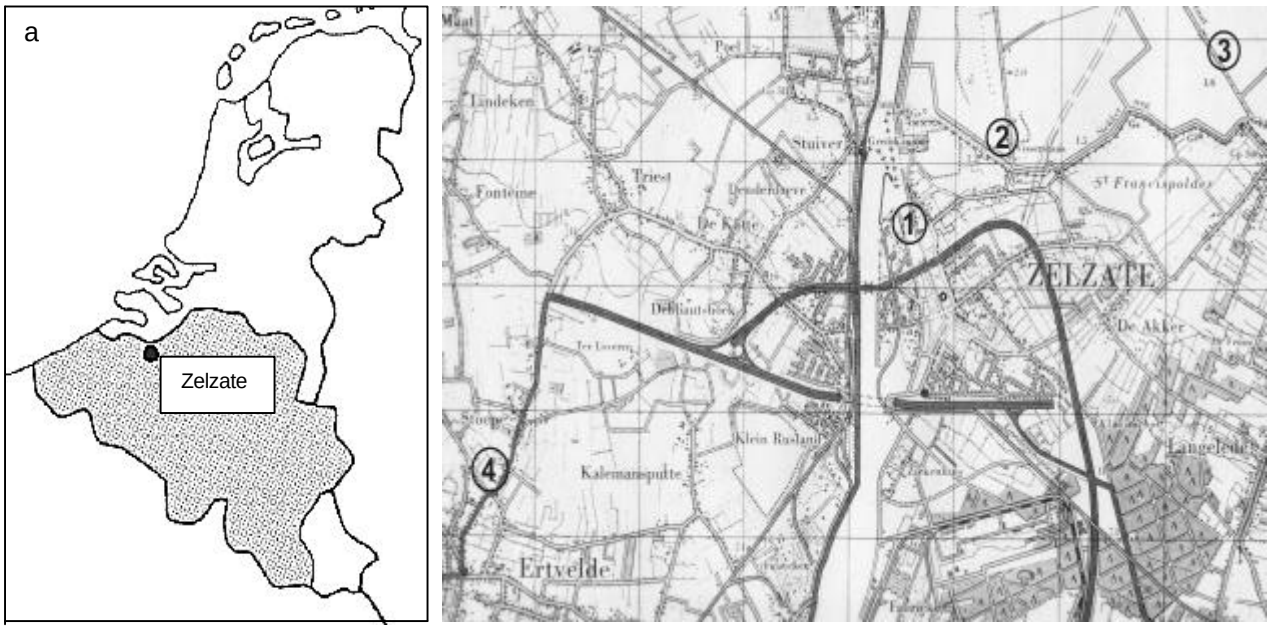

Figure 1. Geographical map of Belgium (a) and of Zelzate (b). The oil refinery and the four sample sites are indicated.

Sample extraction, cleanup and analysis

Soil samples were homogenized and divided into three subsamples per site. The subsamples (2 g) were refluxed with 50 ml MeOH and and 50 ml hexane for four hours. After addition of 30 ml MeOH, the hexane phase was transferred to glass tubes and concentrated to 1 ml under a stream of nitrogen. Cleanup was done on silica gel columns (Baker, Spe, 3 ml), which were pre-eluted with 2 ml of hexane. After addition of the samples, the columns were eluted with 8 ml hexane. The first ml of the eluate was discarded, the next 7 ml were collected, concentrated and the solvent was changed to ACN. Recoveries of this method were > 80 % for all PAHs. The samples were extracted after 6 months of storage. To estimate the loss of compounds during storage, the samples were again extracted and analysed after 27 months of storage.

Plant samples (10 g) were frozen with liquid nitrogen and ground. The extraction and cleanup method are described in **Chapter 5**. In short, 45 ml of methanolic KOH were added to the ground plant material. Subsequently, the mixture was refluxed for 30 minutes. Cyclohexane (100 ml) was added and this was refluxed for 10 minutes. The cyclohexane fraction was concentrated and the solvent was changed to MeOH. Cleanup of the samples took place on Serdolit columns. The columns were eluted with ethanol and pentane, and subsequently with ACN. The ACN fraction was analysed. Recoveries of the PAHs varied from 72 to 90 %, except for BkF and BaP, which amounted to 64 ± 14 and 47 ± 12 %, respectively. Procedural blanks were determined by extraction and cleanup of 45 ml MeOH/KOH (n = 3).

HPLC-analysis of both soil and plant samples was performed as described in **Chapter 4**. BghiP could not be determined quantitatively, due to the presence of an interfering peak of an unknown substance in the chromatogram. PAH-concentrations were calculated by correcting for recovery percentages and subtracting the average amount measured in procedural blanks (extraction and cleanup of pure solvent). The presence of the PAHs was verified by GCMS-analysis (Varian 3400 CX GC fitted with a 30 m J&W DB-5 MS column, coupled with a Saturn 2000 ion trap MS, operating in the SIS-cluster mode).

Results and discussion

Total PAH-concentrations

The total PAH-concentrations for the 7 PAHs in the samples from site 1 are very high, up to 300, 8 and 2 $\mu g \cdot g^{-1}$ DW for soil, *P. major* and grass respectively (Figure 2). The presence of the identified PAHs was confirmed by GCMS-analysis (site 1: all PAHs, site 2-4: only PAHs with MW 178 and 202 could be detected). The PAH-concentrations in the soil samples from site

1 are about two orders of magnitude higher than those in other soils in the vicinity of human activities, which in general amount about 1-10 µg·g^{-1} DW (Table 1).

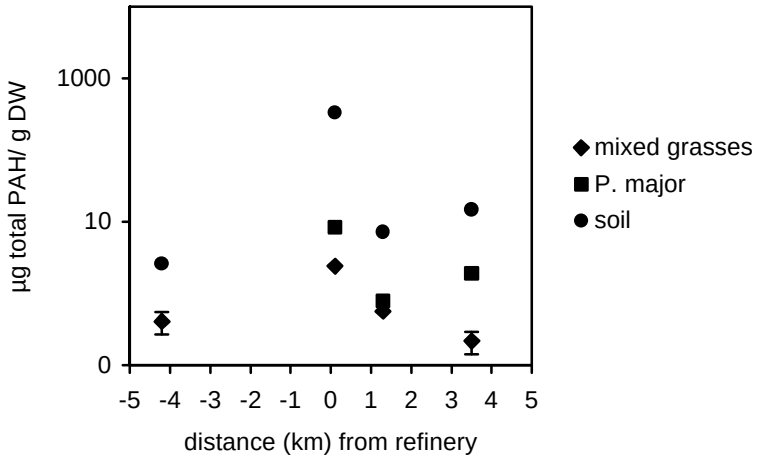

Figure 2. Total concentration of 7 PAHs in leaf samples of grass (diamonds), *P. major* (squares) and soil (circles), collected at different distances in north-east direction from the oil refinery in Zelzate. Error bars represent standard deviations of four samples and when not shown, are smaller than the markers.

Table 1. Total concentrations of PAHs in soils (µg·g^{-1} DW) in urban areas and in the vicinity of human (industrial) activities

location	*number of PAHs*	*soil depth*	*total PAH concentration in soil (µg·g^{-1} DW)*	*reference*
highway (CAN)	17	surface	1.4	(Wang and Meresz 1982)
motorway (UK)	7	0-4 cm	20 at 1 m 4-8 at 600 m	(Butler *et al.* 1984)
highway (USA)	14	0-5 cm	3	(Yang *et al.* 1991)
urban (JAP)	8	0-3 cm	1.3 ± 0.8	(Spitzer and Kuwatsuka 1993)
chemical plant (AUS)	18	0-5 cm	0.3 - 79	(Weiss *et al.* 1994)
urban (CH)	16	0-20 cm	11 ± 12	(Niederer *et al.* 1995)
urban (UK)	16	0-10 cm	2.7 ± 0.5	(Meharg *et al.* 1998)
polyprop. fire (UK)	16	0-10 cm	12 -18	(Meharg *et al.* 1998)

The measured concentrations in the plant samples from site 1 (Figure 2) are within the concentration range in plants from other urban and industrial areas, varying roughly from tens of $ng \cdot g^{-1}$ to 14 $\mu g \cdot g^{-1}$ DW for 10-16 PAHs (Table 2). The variation in the measured plant concentrations (Table 2) is large, arising from differences between species, differences between sampled plant organs (needles, leaves and fruit) and differences in sample treatment. For example, washing of plant material has a large influence on concentrations of particle-bound PAHs (see **Chapter 5**).

Table 2. Total concentrations of PAHs plants ($ng \cdot g^{-1}$ DW) in (sub)urban areas and in the vicinity of human (industrial) activities.

location	*number of PAHs*	*plant*	*total PAH concentration in plant ($ng \cdot g^{-1}$ DW)*	*reference*
highway (S)	16	lettuce	17-90	(Larsson and Sahlberg 1982)
highway (CAN)	17	onions[a], beet[a] tomatoes[a]	10-1900[b]	(Wang and Meresz 1982)
alu. smelter (S)	16	lettuce	300-920	(Larsson and Sahlberg 1982)
highway (S)	16	kale	500 (at 50 m)[b] 5000 (at 10 m)[b]	(Brorström-Lunden and Skärby 1984)
busy city street (S)	16	kale	5000 (at 50 m)[b] 14000 (at 10 m)[b]	(Brorström-Lunden and Skärby 1984)
urban (USA)	10	pine	800-1600	(Simonich and Hites 1994b)
urban (USA)	10	sugar maple	500-1100	(Simonich and Hites 1994b)
urban (I)	10	bay tree[a]	73-880	(Lodovici *et al.* 1994)
(sub)urbans (UK)	16	pine	20-3100[c]	(Tremolada *et al.* 1996)
suburban (USA)	18	maple	510 ± 100	(Wagrowski and Hites 1997)
urban (USA)	18	maple	1600 ± 210	(Wagrowski and Hites 1997)
urban (Germany)	13	kale	1000-5000	(Franzaring 1997)
urban (UK)	16	grass	153 ± 8	(Meharg *et al.* 1998)
polyprop. fire (UK)	16	grass	2400[d]	(Meharg *et al.* 1998)
(sub)urban	8	plantain	200-1700	**Chapter 5**
industrial (GR)	16	various vegetables[a]	25-239	(Kipopoulou *et al.* 1999)

[a] washed leaves; [b]original concentrations expressed in $ng \cdot g^{-1}$ FW, converted to $ng \cdot g^{-1}$ DW assuming a FW/DW of 10; [c]concentration correlated to number of inhabitants; [d]highest concentration

The measured total concentrations in the plant samples from site 1 represent the sum of 7 PAHs. These 7 include the most abundant PAHs (PHEN and FLUO) and the most carcinogenic (BaP). In general, other studies take more PAHs, mostly 16, into consideration. When compared to the sum of concentrations of the seven PAHs selected in this study (i.e. total

concentrations for 7 PAHs, determined in other studies), the total amount for the 16 PAHs is about twice as high as for the selected 7 (*e.g.* Larsson and Sahlberg 1982, Kipopoulou *et al.* 1999). Therefore, it can be assumed that the concentrations in the samples from Zelzate are at the high end of the concentration range reported for urban and industrial areas. Hence, it is likely that the PAHs measured in the samples from site 1 mainly originated from the oil refinery in Zelzate.

The highest concentrations are measured in the soil samples. This was to be expected, as soil accumulates PAHs for a long period of time, whereas exposure of plant organs is just as long as their lifetime (Niederer *et al.* 1995, Meharg *et al.* 1998). The measured concentrations in the soil represent minimum values, as the soil samples were stored at 10°C for 6 months before they were extracted and analysed. During this period, compounds may be lost from the soils by biodegradation and/or evaporation (although the latter was minimised by securely closing the flasks). After 27 months of storage, about 23 % of the amount of the PAHs determined after 6 months was still present in the soil samples. Assuming first order kinetics, this implies that at the time of sampling the concentrations were a factor of ~1.6 higher than those determined after 6 months of storage. This indicates that during the first 6 months the loss due to biodegradation/evaporation was about 37%.

Concentrations in samples from the other sites in Zelzate are a factor 10-30 lower than those measured in samples from site 1 (Figure 2). Apparently, a large part of the emitted compounds is deposited close to the source (particle-bound PAHs) and/or rapidly diluted in the atmosphere (gaseous PAHs). The concentrations in the soil (3-14 $\mu g \cdot g^{-1}$) from sites 2-4 are still at the high end of the range for industrial areas (Table 1). Therefore, it is likely that the levels in sites 2-4 are all increased by the oil refinery. Concentrations in the plants (0.2–1.9 $\mu g \cdot g^{-1}$) are not exceptionally high compared to other reported values (Table 2). The concentrations in soil and plants from sites 2 and 3 are similar to those from site 4, although the latter is in the opposite direction of the prevailing wind (Figure 2). As in the south-west direction of site 4 large industrial activities are absent within close distance, it appears that significant exposure from the oil refinery in the opposite direction of the prevailing wind also occurs.

PAH-profiles

As was mentioned above, ~37% of the PAHs was lost from the soil samples during storage. As a consequence, the PAH-profile may have been changed. However, the concentrations of all compounds decreased at about the same extent at the different sites, resulting in similar profiles after 27 months of storage, compared to a storage time of 6 months (Figure 3). This indicates that, although the total concentrations in the soil samples have been

decreased during 6 months of storage, the measured profiles are still representative of the profiles at the sampling time.

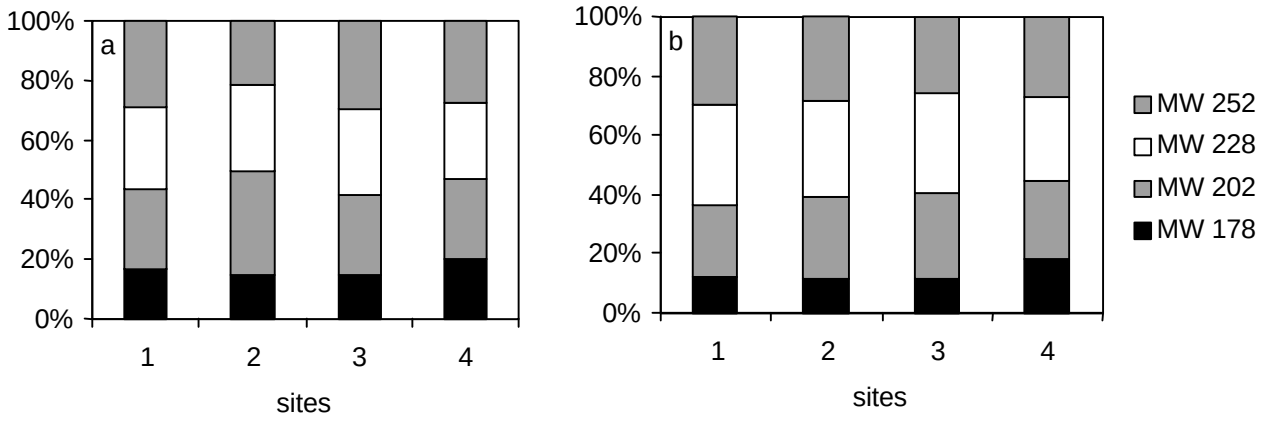

Figure 3. Distribution of PAHs in soil samples from the different sites, measured after 6 (a) and 27 months (b) of storage. PAHs are categorised into groups according to their molecular weight (MW). The location of the sites 1-4 is shown in Figure 1.

The PAH profile in the soil samples is similar for all sites (Figure 3a, 4), with all groups of PAHs having a similar contribution to the total PAH-concentration. However, this is not the case for the plant samples. While for sites 1 and 2 the profiles of the plant samples are similar to that of the soil samples, a different profile is found for *P. major* at site 3 and for grass at site 4 (Figure 4). In these cases, the fraction of PAHs with MW 178 and 202 is relatively high, while PAHs with MW 228 and MW 252 contribute to a lower amount. So, the profile of the two plants samples has changed to a higher contribution of low MW PAHs. In contrast, the grass samples at site 3 have the same PAH-distribution as the samples (from both soil and plant) from site 1 and 2. The difference between the grass and the *P. major* samples will be discussed below (*Species differences*).

The phenomenon of the changing profiles in plant samples at different distances from the source has previously been described by Meharg *et al.* (1998). This author measured PAHs in grass and soil at different distances from a large polypropylene fire and found that high MW PAHs were deposited to plants at smaller distances from the fire (a narrow band at 3 km) than low MW PAHs (3.2 to 4.5 km and further). This was explained by the difference in the behaviour of particle-bound and gaseous PAHs, with particles being deposited closer to the source. Just as in the present study, this trend was not present in the PAH profiles of the soil samples. Meharg *et al.* (1998) argue that the principal mechanism of deposition for soil was deposition of particles for all compounds, due to high amounts of particles originating from the

fire. In contrast, for plants, vapour partitioning was the main deposition route for the PAHs. Possibly, loss of particles from the leaves by blow-off or wash-off may have been responsible for the difference between plants and soil. In the case of an oil refinery, just as during a fire, it may be that large amounts of particles are emitted, which may explain the similar behaviour of the PAHs as in the study of Meharg *et al.* (1998).

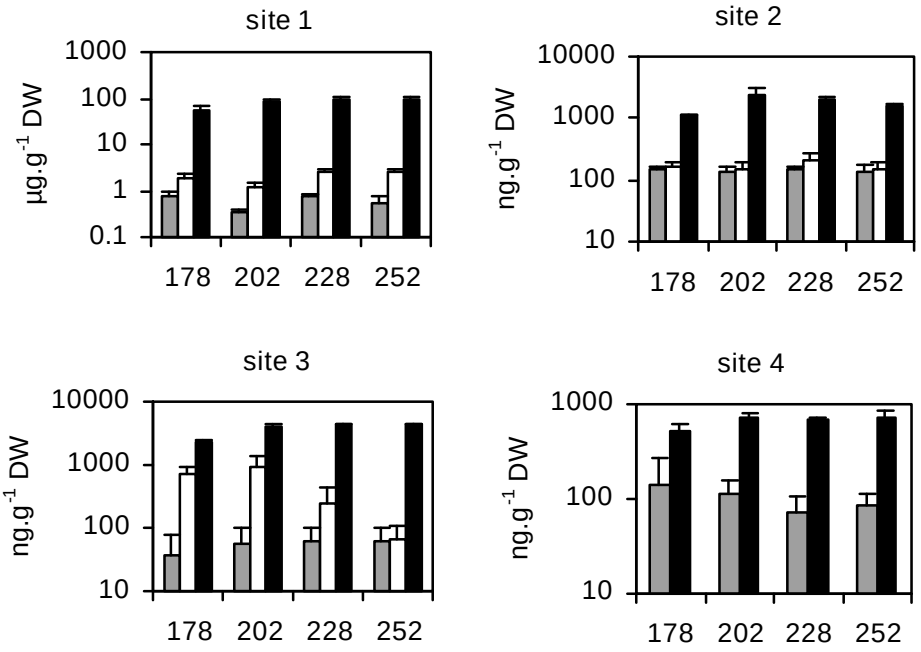

Figure 4. PAH concentrations in leaf samples of grass (hatched bars), *P. major* (white bars) and soil (black bars) collected in Zelzate. The location of the sites 1-4 is shown in Figure 1. PAHs are categorised into groups according to their molecular weight (MW). Error bars represent standard deviations of three (soil) or four (plants) samples, and when not shown, are smaller than the markers.

Species differences

The total PAH-concentrations in *P. major* are higher than the concentrations in the mixed grass samples (Figure 2). However, the difference between the two plants is not constant. The concentration ratio *P. major*/grass is 3.5, 1.2 and 8.8 for the sites 1, 2 and 3, respectively. Furthermore, as mentioned above, the PAH-profiles are not always similar for the two plants: At site 3, the PAH-profile of *P. major* is shifted to a relatively large contribution of low MW PAHs, whereas for grass this shift only occurs at site 4.

The differences in PAH-concentrations are caused by differences in plant characteristics of the two species. SOCs are deposited to plants by three different deposition mechanisms, one of each dominating at a different range of the octanol-air partition coefficients (K_{oa}) of the

compounds (McLachlan 1999). With increasing K_{oa}-values, the dominant deposition mechanisms are: equilibrium partitioning, kinetically limited gaseous deposition and particle-bound deposition (McLachlan 1999). Each deposition mechanism is influenced by specific plant characteristics. The content and composition of plant lipids is the most important plant property in equilibrium partitioning (Simonich and Hites 1995a, Böhme *et al.* 1999). In cases where equilibrium has not been approached, the age of the leaves, the leaf area and the aerodynamic surface roughness of the plant are key factors in determining the SOC concentrations in plants (**Chapter 2**). For particle-bound deposition, the roughness of the leaf surface, *e.g.* the presence of leaf hairs (**Chapter 4**) and the orientation of the leaves are also of importance (Welsch-Pausch and McLachlan 1996, Böhme *et al.* 1999). In the paragraphs below, the plant characteristics which are important for the three deposition mechanisms will be discussed to explain the difference between the PAH-concentrations in P. major and grass.

Equilibrium partitioning probably has occurred for the PAHs with MW 178 and 202 (Böhme *et al.* 1999), if they were present as gases. It is not likely that the difference between the PAH-concentrations of the two plants is caused by differences in lipid content or composition. Firstly, the lipid contents of grass and *P. major* are similar. Although the lipid content was not determined in this experiment, measurements in other experiments indicate that grass has a lipid content of about 0.3-0.8 % (Tolls and McLachlan 1994, Böhme *et al.* 1999) and that of *P. major* is ~0.3% (**Chapter 4**). Secondly, although the lipid composition of the sampled plants was not investigated, other studies show that they are probably comparable as well. The lipid composition is assumed to determine the slope of the plot of concentration in vegetation/gaseous concentration in air versus $\log K_{oa}$ (under equilibrium conditions), (Kömp and McLachlan 1997a). In two studies, the slopes of these plots for grass and *Plantago lanceolata* were similar (Kömp and McLachlan 1997a, Böhme *et al.* 1999). As the lipid composition of *P. lanceolata* and *P. major* are alike (**Chapter 4**), it is not likely that the difference in plant concentrations is caused by a different lipid composition.

The specific leaf area (SLA) of the plants, important for kinetically limited gaseous deposition and deposition of particles, is comparable as well. The average SLA of *P. major* was 5.8 ± 0.8 $m^2 \cdot kg^{-1}$ (two sides of leaves). The SLA of the grass samples was not measured, but for *Lolium multiflorum* it was determined to be 4.4 $m^2 \cdot kg^{-1}$ (Tolls and McLachlan 1994), while that of *Lolium perenne* is about 5.0 $m^2 \cdot kg^{-1}$ (Bakker, unpublished results).

As both species have smooth leaf surfaces, differences in particle-bound deposition cannot be explained by a difference in roughness of the leaf surface. However, the architecture of the two plant species is very different. *P. major* likely has a higher aerodynamic surface roughness than grass, as the large leaves are standing relatively free and high in the air. Leaves of grass form a dense canopy and therefore may create less aerodynamic turbulence, resulting in lower PAH-concentrations. In addition, as leaves of *P. major* are mainly horizontally oriented, they may be more efficient in scavenging particles than vertically oriented grass leaves. This may

explain the larger concentrations of high MW PAHs in *P. major* (and also the higher concentrations of the other PAHs, in case of a high particle-bound fraction of all PAHs, as was suggested above).

However, the orientation of the leaves cannot explain the shift of the PAH-profile for *P. major* at site 3. At site 3, *P. major* contains a large fraction of low MW PAHs, in contrast with grass. This indicates that the dominant deposition mechanism at site 3 has changed to gaseous deposition for *P. major* and remains particle-bound deposition for grass, which is in contrast with the leaf orientation explanation. The cause of this phenomenon remains unclear.

Finally, differences in leaf age of the plants may be an explanation for the difference in PAH-concentrations. Leaf age plays a role in kinetically limited gaseous deposition and particle-bound deposition: older leaves have been exposed for longer times and therefore can have reached higher concentrations. However, the age of the leaves in this experiment is unknown, so no conclusions can be drawn on this.

To summarize, the large differences between the PAH-concentrations of *P. major* and grasses at sites 1 and 3 cannot be explained by the relatively small differences in lipid content and composition, SLA or roughness of the leaf surface. Hence, it appears that aerodynamic surface roughness, leaf orientation and leaf age most likely are the plant characteristics responsible for the differences in PAH-concentrations. The different profiles for *P. major* and grass show that the dominant deposition mechanism for the two plants changes at different distances from the source. The cause of this difference remains unclear.

As the concentration ratio between the plants is not constant between the sites, it is not possible to apply a conversion factor to the vegetation to precisely predict the concentration in one species from the known concentration of the other. This conclusion was also drawn in a study on the accumulation of chlorinated hydrocarbons in pine needles and lichens, where the differences amounted from a factor of 3 up to >50 (Ockenden *et al.* 1998). This large difference was most likely due to the extreme difference in age of the plants (2 years for the pine needles and 25-50 years for the lichens). The differences between the species in this study are at maximum a factor 8.8. In many other studies only small differences (< a factor of ~8) between SOC concentrations in plants have been found (**Chapter 2**). Hence, a rough estimate of the SOC-concentrations can be made from known concentrations of other species. For precise determinations, however, the sampling strategy after incidents or close to a continuous source still needs to be focussed on all species of interest.

Acknowledgement

We are grateful to Mr. De Meijer from the town council of Zelzate for the kind cooperation and for giving us the opportunity to collect samples from the closed city park. We acknowledge Pieter Sleijpen for his help with the collection of the samples and Frans Busser for the GCMS analyses.

9

EVALUATION
of predictive models
WITH respect to
plant characteristics

Abstract

Two predictive models, one for dry atmospheric gaseous deposition (Trapp and Matthies 1995) and one for atmospheric particle-bound deposition (McLachlan 1999) of semivolatile organic compounds to plants are evaluated. The variation in plant characteristics and its effect on the predicted plant concentrations are discussed. For dry gaseous deposition, K_{pa}-values and elimination rate constants calculated with the model (Trapp and Matthies 1995) are compared to experimentally determined values. Under equilibrium conditions, calculated K_{pa}-values are relatively similar to laboratory determined values. Difference with field-determined values are larger (a factor of 5-20 for most plants). For non-equilibrium gaseous deposition the model predictions are uncertain. This is due to the large variation in the conductance under influence of the environmental conditions and the plant characteristics. It is recommended that the deposition of particle-bound SOCs is included in the model for the prediction of concentrations in cattle feed. As the variation in the particle-bound deposition velocities of SOCs is large, these predictions will also have a high degree of uncertainty. For the prediction of concentrations in plants from “continuous” emissions, the use of plant specific parameters in models has little advantage, because (1) plant parameters are not constant, but dependent on the environmental conditions and (2) the environmental conditions also have a large effect on the uptake/elimination rate and/or equilibrium concentrations. In the case of incidents, plant specific input values may increase the accuracy of the predictions. As the effect of environmental conditions on plant concentrations is quantitatively unknown, concentrations in plants cannot be estimated precisely. For an accurate risk assessment (within a factor of ~10) measured concentrations in plants are therefore necessary.

Introduction

Various models have been developed to describe the uptake of organic contaminants by plants, (*e.g.* Briggs *et al.* 1982, Sabljiæ *et al.* 1990, Paterson *et al.* 1994, Riederer 1994, Trapp and Matthies 1995, McLachlan 1999). Some of these models consider both uptake of the contaminant via the plant roots and the atmospheric deposition to aerial plant parts (Paterson *et al.* 1994, Trapp and Matthies 1995), while others only focus on the latter process (Sabljiæ *et al.* 1990, Riederer 1994, McLachlan 1999). In a few models the uptake kinetics are considered (Trapp and Matthies 1995, McLachlan 1999), which is essential for prediction of concentration in vegetables, as they are in the field for relatively short time periods. In the models, a number of plant characteristics, such as the total lipid content, the surface area to volume ratio and the growth rate, are incorporated. Nevertheless, often values for a "standard" plant, instead of plant specific values, are employed.

Predictive models can be used to assess the risks of contaminants in our food for human health. For example, the European Union System for the Evaluation of Substances (EUSES, Vermeire *et al.* 1997) estimates concentrations of SOCs and other chemicals in the food of European citizens, using emission data. One of the parts of EUSES is the estimation of concentrations of organic contaminants in plants. The risk assessment of EUSES is based on an average consumption pattern and therefore, the use of average plant parameters may be justified. However, the values that are used in the model may not represent the "average plant" that is consumed. In case of risk assessment of incidents (*e.g.* large fires or accidental emission of chemicals) there may be a need to estimate concentrations in specific plants (such as grass for cattle feed). In this situation the use of specific plant characteristics may be necessary for an appropriate risk assessment.

In this chapter, the prediction of plant concentrations originating from dry gaseous deposition by the model of Trapp and Matthies (1995) is evaluated, as this is a kinetic model and incorporated in EUSES. The model evaluation will focus on the plant characteristics. In addition, a model for prediction of particle-bound deposition will be discussed.

Modelling dry gaseous deposition with EUSES

The model of Trapp and Matthies (1995) employs a simple one compartment first order kinetic model to describe dry gaseous deposition of SOCs in plants (**Chapter 2**). As the plant-air partition coefficient K_{pa} can be represented by $L \cdot K_{oa}^{n}$ (**Chapter 2**), the general equation of the model (equation 4, **Chapter 2**) can be given as:

$$C_p = K_{pa} \cdot C_a \cdot (1 - \exp[-k_2 \cdot t]) = L \cdot K_{oa}^{n} \cdot C_a \cdot (1 - \exp\left[-\frac{A \cdot g \cdot t}{V \cdot L \cdot K_{oa}^{n}}\right]) \qquad (1)$$

where C_p is the concentration in the plant ($mol \cdot m^{-3}$), K_{pa} is the plant-air partition coefficient ($m^3 \cdot m^{-3}$), C_a is the concentration of gaseous compounds in air ($mol \cdot m^{-3}$), k_2 is the elimination rate constant (h^{-1}), t is the exposure time (h), L is the lipid volume fraction, K_{oa} is the octanol-air partition coefficient, n is the slope of the $logK_{pa}$-$logK_{oa}$ plot, A is the surface area of the plant (m^2), g is the conductance or deposition velocity ($m \cdot h^{-1}$) and V is the volume of the plant (m^3).

Trapp and Matthies (1995) add growth dilution to their model as a first order kinetic elimination process. Also, metabolism and photodegradation of SOCs are included in the model. The total elimination rate constant is then

$$k_2 = \frac{A \cdot g}{V \cdot L \cdot K_{oa}^{\ n}} + k_{growth} + k_{metab.} + k_{photo.} \qquad (2)$$

where k_2 is the total elimination rate constant (h^{-1}) and $k_{growth,}$ $k_{metab.}$ and $k_{photo.}$ are rate constants (h^{-1}) for growth, metabolism and photodegradation, respectively. However, the metabolism and photodegradation rate are not known for most compounds and EUSES uses the value 0 for $k_{metab.} + k_{photo.}$ as a worst case approach.

An additional elimination process is the erosion of wax layers (Horstmann and McLachlan 1996). This is not incorporated in the model of Trapp and Matthies. Since this process is hardly investigated, elimination rates of erosion are not known. Therefore, this parameter will not be included in the present model evaluation.

In the following sections the variation of plant characteristics and its effect on equilibrium and non-equilibrium partitioning of gaseous SOCs will be discussed. In addition, the values calculated by the model for K_{pa} and k_2 will be compared with experimentally determined values.

Equilibrium partitioning

Plant characteristics

For compounds with low K_{oa} values, the dominating deposition mechanism is equilibrium partitioning (McLachlan 1999). When growth is not considered, the model is simplified to:

$$\frac{C_p}{C_a} = K_{pa} = L \cdot K_{oa}^{\ n} \qquad (3)$$

In this equation, the plant characteristics are the lipid volume fraction L and the slope of the $logK_{pa}$-$logK_{oa}$ plot n.

Most food plants (animals and humans) are herbaceous plants (Polder *et al.* 1997). The values for the lipid volume fraction of herbaceous plants reported in the literature range from ~ 0.1-1.7 % (Table 1). In EUSES, for the lipid volume fraction a value of 1 % is used.

Note that the lipid content, just as any other plant parameter, can be affected by environmental conditions, such as temperature.

Table 1 Lipid fraction, surface area to volume ratio and density of (leaves of) some plants

plant	*lipid volume fraction (%)*	*A/V* [a] *($m^2 \cdot m^{-3}$)*	*plant density ($kg \cdot m^{-3}$)*	*reference*
kale	0.28	500	925	(Flindt 1988)
lettuce	0.16	2440	610	idem
kale	1.2	4800	830	(McCrady 1994)
pepper	1.1	8000	870	idem
grass	1.2	17500	810	idem
ryegrass	0.3	7200	820	(Tolls and McLachlan 1994)
soy bean, leaves	1.7	n.r.[b]	750	(Tam *et al.* 1996)
spinach	0.23	1050	630	(Sprenger Institute, 1997)
endive	0.18	820	735	idem
ryegrass	0.68[c]	14700	n.r.	(Böhme *et al.* 1999)
creeping thistle	0.44[c]	4000	n. r.	idem
dandelion	0.41[c]	8800	n. r.	idem
ribwort plantain	0.23[c]	6300	n. r.	idem
yarrow	0.46[c]	5800	n. r.	idem
lady's mantle	0.66[c]	8100	n. r.	idem
sunflower, leaves	0.35[c]	5300	n. r.	idem
autumn hawkbit	0.45[c]	6100	n. r.	idem
white clover	0.30[c]	6600	n. r.	idem
corn, leaves	0.54[c]	8600	n. r.	idem
great plantain, leaves	0.01[c, d, e]	4700	n. r.	present study
ribwort plantain, leaves	0.01[c, d, e]	3900	n. r.	present study
hoary plantain, leaves	0.01[c, d, e]	5000	n. r.	present study

[a] it is not clear whether the area of one side or two sides of the leaf are reported except for Tolls (1994), Böhme (1999) and the present study (two-sided leaf areas) ; [b] not reported; [c]assumed lipid density of 1000 $kg \cdot m^{-3}$ (Baur *et al.* 1999); [d]assumed plant de7nsity of 850 $kg \cdot m^{-3}$; [e] extractable epicuticular wax only

The lipid volume fraction is calculated with the amount of the extractable plant lipids. However, this method gives only a rough estimation of the total lipid volume fraction, since hydrophobic compounds may also accumulate in the non-extractable cutin (Umlauf *et al.* 1994b). It has been suggested to determine the volume of the cuticle instead of that of the extractable lipids (Böhme *et al.* 1999). Böhme estimated this factor to be 0.1-1.6 % for four of the test species used in his study, while the variation in extractable lipids of those species was only a factor of 2. Therefore, the variation in cuticular volume may be higher than that in extractable lipids.

The other plant parameter in equation (3) is the constant n, which represents the similarity of the lipids to octanol. The constant n varies from ~0.3-1.15 for different plants (Table 1, **Chapter 2**). In EUSES a value of 0.95 is used for n. It should be noted that this n is different from the one used in equation (3), as the K_{pa} in EUSES (and in Trapp and Matthies 1995) is represented by $L \cdot K_{ow}^{n}/K_{aw}$ rather than by $L \cdot K_{oa}^{n}$.

The value of n can have a large influence on the predicted plant concentrations, especially for the compounds with large K_{oa} values. This is confirmed by a sensitivity analysis (Jager and Hamers 1997). It can be calculated that, while for compounds with $\log K_{oa}$ of 2 the difference between n = 0.3 and n = 1.15 leads to 2 orders of magnitude difference in the plant concentration; for compounds having a $\log K_{oa}$ of 4 the difference is 4 orders of magnitude.

Comparing calculated with experimentally determined K_{pa} values

K_{pa} values determined in the laboratory

Polder *et al*. (1997) compared experimentally determined K_{pa} values (measured under laboratory conditions) for herbaceous plants to K_{pa} values which were calculated with the model of Trapp and Matthies. The variation between experimental and calculated values was within a factor of 5 for most compounds (Polder *et al.* 1997). Below, more recently measured K_{pa} values are compared to calculated ones. In this comparison, growth was not considered, as laboratory experiments are mostly short lasting experiments in which growth dilution is assumed to be negligible. The measured K_{pa} values of some chlorinated hydrocarbons and PAHs in ryegrass (Tolls 1994; not included in Polder *et al*. 1997) are about 4 times higher than the calculated values (Figure 1). Differences between K_{pa} values from PCBs of various species (Kömp and McLachlan 1997a) and calculated K_{pa} values are within a factor of 7, while those of the present study (**Chapter 7**) differ a factor of about 3 from the calculated values (Figure 1).

K_{pa} values determined in the field

In field experiments, plants are mostly exposed for long time periods (weeks to months). Consequently, when calculated K_{pa} values are compared to experimentally determined values in the field, the growth of the plants needs to be included in the model. The growth rate is dependent on the species and on the environmental conditions. Trapp and Matthies proposed a growth rate of 0.035 d^{-1} (0.0015 h^{-1}), which means a half-life of the compound (*i.e.* growing of the plant to twice the original volume) of 20 days. This value is employed in EUSES and therefore will also be used here.

Böhme *et al.* (Böhme *et al.* 1999) found that for each of the ten plant species studied, five compounds approached an equilibrium between the plant and the air: penta- and hexachlorobenzene and three small PCBs. The K_{pa} values of these compounds ($m^3 \cdot m^{-3}$; assumed plant density 850 $kg \cdot m^{-3}$) are compared to the calculated K_{pa} values (Figure 2).

For most plant species the measured K_{pa} is a factor 5-20 higher than the calculated K_{pa}. For the “extreme plants” yarrow and sunflower, which had high concentrations compared to the other eight species (Böhme *et al.* 1999, see also **Chapter 2**), the difference is much larger: a factor 80-100 for the chlorobenzenes and 15-40 for the PCBs.

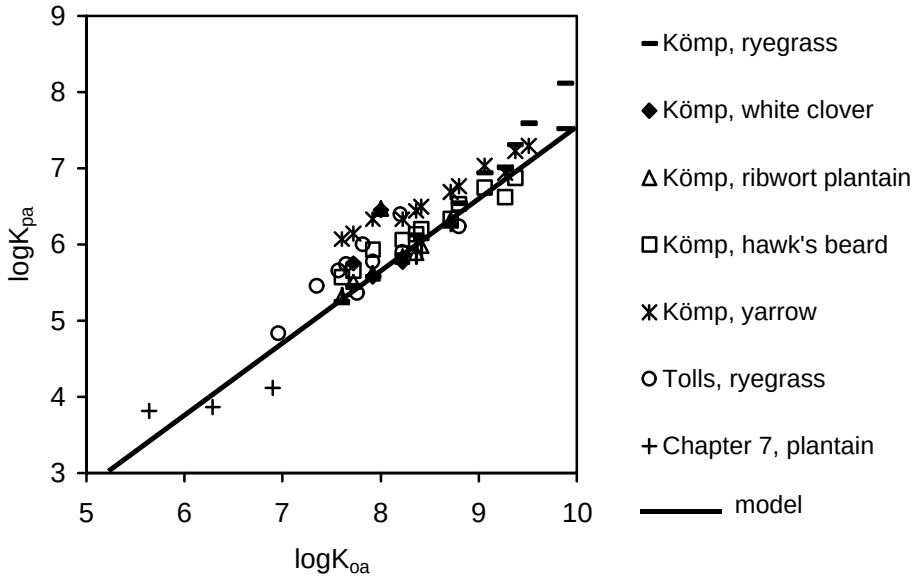

Figure 1 $LogK_{pa}$ determined in laboratory (data from Tolls and McLachlan 1994, Kömp and McLachlan 1997a and Chapter 7) and calculated according to the model of Trapp and Matthies (1995) -assuming negligible growth- versus $logK_{oa}$ of the compounds.

Thomas (Thomas *et al.* 1998) measured PCBs in air and pasture at a windy site and showed that for all PCBs equilibrium had been reached within two weeks. The values for K_{pa} (transformed to $m^3 \cdot m^{-3}$ with an assumed DW/FW ratio of 15% and assumed plant density of 850 $m^3 \cdot kg^{-1}$) are 7 to 25 times higher than the calculated K_{pa} values (Figure 2).

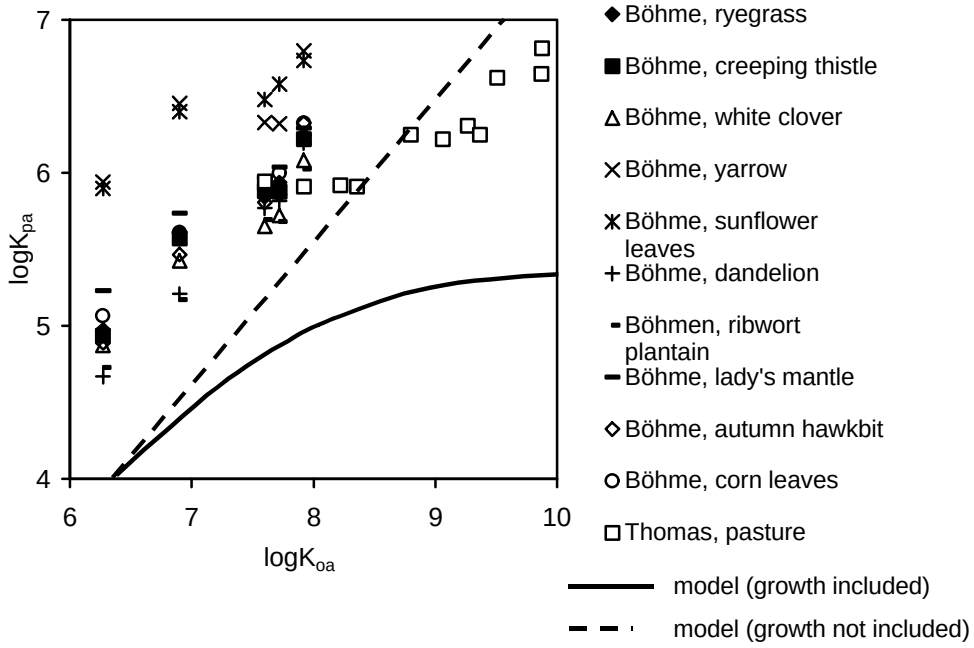

Figure 2 K_{pa} values measured in field studies (Thomas *et al.* 1998, Böhme *et al.* 1999) and calculated according to Trapp and Matthies (1995) plotted versus $logK_{oa}$ of the compounds.

The finding that the measured K_{pa} values are consistently higher (about one log unit) than the calculated values (Figure 2) cannot be explained by the temperature difference between the field-measured K_{pa}'s (average temperature likely lower than 25 °C) and the calculated K_{pa}'s (using K_{oa} values determined at 25 °C). Although the K_{oa} values increase with decreasing temperature, the calculated K_{pa} values do so as well and therefore, the position of the model curve in Figure 2 will not significantly change.

The consistent difference between the measured and the calculated K_{pa} values must therefore originate from the choice of one of the other model parameters. Growth dilution is not expected to be the cause, as this has only an effect on very hydrophobic compounds (see below). As the difference between modelled and measured K_{pa} values is small for laboratory experiments (Figure 1), the large difference under field conditions is likely caused by a parameter for which the value under field conditions is different from the one under laboratory conditions. As the slope of the log-log plot for several plants in the field (Böhme *et al.* 1999) is similar to that in the laboratory (Kömp and McLachlan 1997a), (see Table 1 in **Chapter 2**), this is probably not the cause of the difference. The lipid content is the remaining parameter, and as this can be affected by environmental conditions such as temperature and humidity (*e.g.* Cape and Percy 1993) it is a likely candidate causing the difference. Hence, the choice of the value of 0.01 for this parameter may be too low.

The effect of growth is only important for the calculated K_{pa} values of the more hydrophobic compounds (Jager and Hamers 1997). An uncertainty analyis indeed showed that the plant concentration of benzo[a]pyrene ($logK_{oa}$ 9.62, calculated from $K_{ow,}$ De Maagd *et al.* 1998 and Henry's Law constant, Mackay *et al.* 1991) is extremely sensitive to k_{growth}: 78% of the variance in the foliar uptake was explained by this parameter (Versluijs *et al.* 1998). The high sensitivity of high hydrophobic compounds to growth is explained by their very slow "clearance". This renders the effect of growth on the total elimination relatively high. If growth is not taken into account, the calculated K_{pa} values for the high PCBs ($logK_{oa} > 8$) are higher, and not lower, than the measured values of Thomas (Figure 2).

Conclusion equilibrium partitioning

The variation in the lipid volume fraction L and the slope of the $logK_{pa}$-$logK_{oa}$ plot n are both large. This can affect the K_{pa}. It has little advantage, however, to use plant specific values for these parameters in the model, as the variation is not only due to species differences, but also due to different environmental conditions. For most plants, the modelled K_{pa} values differ only a factor 5-7 from the experimentally determined values in the laboratory. The differences between calculated and measured K_{pa} values in the field is larger: about a factor 5-20 for most plants, up to a factor of 80-100 in some cases. This may becaused by too low values for the

lipid content. For K_{pa} values determined in field experiments, growth is significant for compounds with high K_{oa} values.

Non-equilibrium gaseous deposition

Plant characteristics

For compounds with higher K_{oa} values ($\log K_{oa}$ >~8), the storage capacity of the plant for the compound is so high that the plant concentrations remain far from equilibrium (McLachlan 1995, 1999). The plant concentration is described by equation (1) and will depend on the value of k_2 and the exposure time. In this case, the essential plant parameters are the growth rate, the surface area to volume ratio A/V and the conductance g.

For the surface area to volume ratio of herbaceous plants, the range found in the literature is 500-17000 $m^2 \cdot m^{-3}$ (Table 1). Trapp and Matthies propose a value for A/V of 2500 $m^2 \cdot m^{-3}$, which is used in EUSES.

The conductance consists of three conductances in series, corresponding with the three steps in the deposition process (transport through the bulk atmosphere, the laminar boundary layer and the cuticle) as described in **Chapter 2**. The total conductance can be described by

$$g_{total} = \frac{1}{\frac{1}{g_{aerodyn.}} + \frac{1}{g_{b.l.}} + \frac{1}{g_{cuticle}}} \qquad (4)$$

where $g_{aerodyn.}$ is the conductance (or deposition velocitiy) in the atmosphere, $g_{b.l.}$ the conductance of the laminar boundary layer and $g_{cuticle}$ that of the cuticle. The aerodynamic and boundary layer conductance represent the “air side conductance” of the deposition process, while the cuticular conductance stands for the “plant side conductance”. For compounds with $\log K_{oa}$>~8, it is assumed that the cuticle conductance is high compared to the other two conductances, so the rate-limiting step is in the atmosphere and not in the plant (McLachlan *et al.* 1995). Consequently, for these compounds the total conductance is determined by $g_{aerodyn}$ and $g_{b.l.}$ and not by $g_{cuticle}$. For compounds with low K_{oa} values ($\log K_{oa}$ < ~4), the rate limiting step is in the cuticle of the plant (McLachlan *et al.* 1995), and $g_{cuticle}$ determines the overall conductance. Below, both the air side and plant side conductance will be discussed in more detail.

Air side conductance

As noted above, the air side conductance consists of the boundary layer conductance and the aerodynamic conductance. The boundary layer conductance can be calculated by the diffusion coefficient of the compound in air divided by the thickness of the laminar boundary layer (Riederer 1994). The latter is largely determined by the plant architecture (shape and size

of leaf) and the wind speed and may vary from less than 1 mm to more than 10 mm (Burrage 1971). The diffusion coefficient in air is dependent on the molar volume of the compound and can be estimated as $1.55/MW^{0.65}$ $cm^2 \cdot s^{-1}$ (Schwarzenbach *et al.* 1993). So, for a compound with a MW of 250, values for the boundary layer conductance are in the range 1.5 – 15 $m \cdot h^{-1}$.

The aerodynamic conductance ($g_{aerodyn.}$) or the aerodynamic deposition velocity is a complex function of the metereological conditions (wind speed) and of the plant architecture (aerodynamic surface roughness), (McLachlan 1999). Although the exact relationship of the aerodynamic surface roughness of a plant with the deposition velocity is unknown, it is clear that its effect can be large. Results of the present research showed that the difference between intact and clipped *P. media* was a factor 9, while difference in plant height was only a factor 3 (**Chapter 6**). Furthermore, in other studies a strong relation between the deposition and the herbage density or leaf area index (which are measures for the aerodynamic surface roughness) has been shown (Witherspoon and Taylor Jr. 1970, Jonas and Heinemann 1985, Heil 1988, Schuepp 1989). However, it is not yet established how the wind speed and the aerodynamic surface roughness effect the conductance quantitatively. As a consequence, the aerodynamic conductance cannot be estimated from theoretical considerations.

Plant side conductance

The conductance of the cuticle consists of two parallel conductances: that of the stomata and that of the cuticular wax (Riederer 1994). The sum of those two conductances gives the total conductance of the cuticle.

The stomata form a pathway for the exchange of organic vapors parallel to that across the cuticle. They are partially or completely closed during periods of water stress and, with most plant species, at darkness (Riederer 1994). The stomatal conductance can be defined as:

$$g_{sto} = \frac{D_{air} \cdot n \cdot a_{sto} \cdot Q}{x_{sto}} \qquad (5)$$

where n is the number of stomata, a_{sto} the fractional area of one open stoma ($n \cdot a_{sto}$ is the portion of the total leaf area made up by stomata when they are all open, ranging from 0.002 to 0.02 for different species), Q is the mean degree of opening of the stomata ($0 \leq Q \leq 1$) and x_{sto} is the effective path length (~30 µm), (Riederer 1994). Assuming that all stomata are open, for a molecule with a MW of 250 a stomatal conductance of ~ 0.72 – 7.2 $m \cdot h^{-1}$ can be estimated.

The conductance of the cuticular wax (g_{wax}) may be estimated by the diffusion coefficient in the wax (D_{wax}) times the air-cuticular wax partition coefficient ($K_{cut.wax/air}$, estimated by K_{oa}^{n}) divided by the pathway through the wax (x_{wax}), (Riederer 1994). According to this calculation method, the cuticular wax conductance increases with increasing K_{oa} of the compounds. For example, assuming an x_{wax} of 10 µm and calculating the D_{wax} from the molar volume by extrapolation of the results of Schreiber and coworkers (Schreiber and Schönherr 1993, Schreiber 1995, Schreiber *et al.* 1996), gives a g_{wax} of $1.2 \cdot 10^{-4}$ $m \cdot h^{-1}$ for tetrachlorobenzene

and of 0.6 $m \cdot h^{-1}$ for benzo[k]fluoranthene (Table 2). This is not in agreement with the results of **Chapter 5** and **7**. In these chapters it was found that the transfer of the compounds to the interiors of the leaves decreased with increasing K_{oa} of the compounds. The discrepancy could be due to the fact that the calculation method as proposed by Riederer (1994) assumes diffusion through a homogeneous medium. However, cuticular waxes may not be homogeneous, but may be considered a porous medium, in which compounds can transfer from one sorption site to another by partitioning from the wax to the air in the pores and back (like in a packed column GC). In such a scenario, the transfer rate decreases with increasing K_{oa} of the compound. At present too few data are available to evaluate which of the proposed mechanisms is actually involved. Hence, a suitable calculation method is presently not known, and consequently, accurate values for the conductance of the cuticular wax can not be estimated.

Table 2 Calculated diffusion coefficients (D_{wax}, $m^2 \cdot s^{-1}$) and cuticular wax conductances (g_{wax} $m \cdot h^{-1}$, calculated as $D_{wax} \cdot Koa^n/10$ µm) for some SOCs

compound	*logK_{oa}*	*D_{wax} ($m^2 \cdot s^{-1}$)*[a]	*g_{wax} ($m \cdot h^{-1}$)*[b]
1,2,3,4-tetrachlorobenzene	5.64[c]	$1.5 \cdot 10^{-16}$	$1.2 \cdot 10^{-4}$
hexachlorobenzene	6.9[c]	$6.9 \cdot 10^{-17}$	$8.9 \cdot 10^{-4}$
phenantrene	7.57[d]	$6.7 \cdot 10^{-17}$	$3.8 \cdot 10^{-3}$
benzo[k]fluoranthene	11.63[e]	$1.4 \cdot 10^{-17}$	$6.0 \cdot 10^{-1}$

[a] calculated with McGowan's characteristic molar volume (Abraham and McGowan 1987) and by extrapolation of the results of Schreiber and Schönherr (1993), Schreiber (1995) and Schreiber *et al.* (1996); [b] calculated according to Riederer (1994); [c] Harner and Mackay (1995); [d] Harner and Bidleman (1998); [e] calculated from Kow and Henry's Law constant (De Maagd *et al.* 1998)

Conductance in EUSES

For the total conductance, EUSES uses the values proposed by Trapp and Matthies (1995) of 0.001 $m \cdot s^{-1}$ (3.6 $m \cdot h^{-1}$), which is an average value calculated from the conductance of the stomatal pathway and that of the cuticular pathway (as calculated according to Riederer 1994). However, it is not clear if the aerodynamic and the boundary layer conductance are considered by the authors. Therefore, the choice of the value of 3.6 $m \cdot h^{-1}$ appears arbitrary. Nevertheless, presently, adequate estimation methods for the calculation of the total conductance are not available, since the relationship of the conductances of the three steps in the deposition process with the plant characteristics and the environmental conditions is not known.

Comparing calculated uptake and elimination constants with experimentally determined ones

As mentioned above (equation 1), the plant concentration under non-equilibrium conditions depends on the elimination rate constant k_2. Below, the values of this constant, calculated with the model of Trapp and Matthies (equation 2) is compared with experimental values from the literature. In this case, also non-herbaceous species have been included, as data on herbaceous plants are scarce. All experimental values are derived in the laboratory, except for the values measured by Kömp (Kömp and McLachlan 2000), which were obtained by placing "loaded" plants outdoors at a rural site.

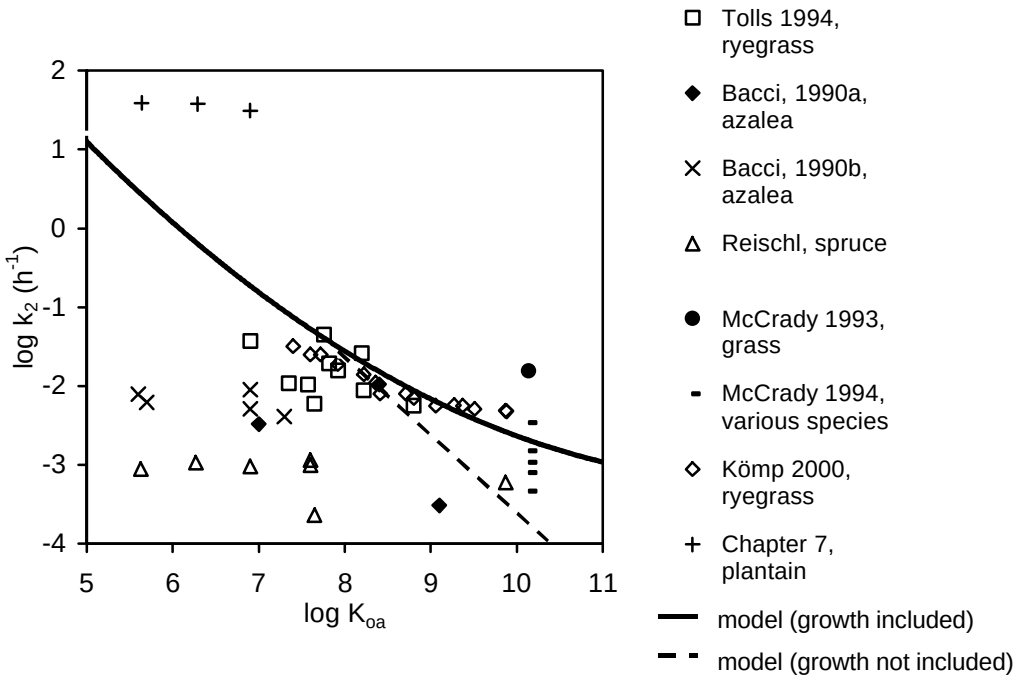

Figure 4 Experimentally determined values and calculated values (Trapp and Matthies 1995) of log k_2 (h^{-1}) plotted versus the log K_{oa} of the compound. Values obtained from (Reischl *et al.* 1989, Bacci *et al.* 1990a, Bacci *et al.* 1990b, McCrady and Maggard 1993, McCrady 1994, Tolls and McLachlan 1994, Kömp 2000). Data (k_1) from McCrady (1994) were divided by K_{pa} values calculated by the model to obtain k_2 values.

The elimination rates calculated with the model are generally higher than the experimentally determined values (Figure 4). The difference between the calculated and the measured values covers a large range: from a 10-100 times overestimation for plantain to an underestimation of 3 orders of magnitude for spruce (Figure 4). This large range is not surprising, as different plant species and different turbulent conditions were used in the experiments and the difference in the conductance therefore may be large. The largest differences between calculated and experimentally determined k_2 values are found for the non-herbaceous species spruce and azalea (Figure 4). For ryegrass, they are relatively small:

varying from a factor of 0.1-2 (Tolls 1994) and of 0.4 to 13 (Kömp and McLachlan 2000). It should be noted here that in the latter study, the errors in the elimination rate constants of the highly chlorinated PCBs were very high (Kömp 2000).

When the leaf area to volume ratio and K_{pa} of the investigated plants is known, the value of the conductance can be estimated from the elimination rate constant k_2. This value varies from 0.5-5 $m \cdot h^{-1}$ (Kömp 2000), 0.3-9 $m \cdot h^{-1}$ (Tolls 1994) and 8-10 $m \cdot h^{-1}$ (McCrady and Maggard 1993, McCrady 1994) for grass, to 4-10 $m \cdot h^{-1}$ for kale, pepper and azalea (McCrady 1994) and 50-80 $m \cdot h^{-1}$ for plantain (**Chapter 7**). The measured conductances cover a range of two orders of magnitude. The value used by EUSES (3.6 $m \cdot h^{-1}$) is in the middle of this range.

Conclusion non-equilibrium dry gaseous deposition

The total conductance is important for the uptake of gaseous compounds under non-equilibrium conditions. However, no quantitative relationships between the conductance and the environmental conditions and/or plant species are known. The other plant characteristics significant for this deposition mechanism, namely A/V and the growth rate, are also dependent on the environmental conditions and the plant species. Measured elimination rate constants cover a range of 3 to 5 orders of magnitude. The values used in EUSES seem arbitrarily selected, leading to elimination rate constants which are generally at the high end of the range of measured values. At present, it seems impossible to calculate accurate elimination rates for all plant species under all environmental conditions. Therefore, when predicting plant concentrations with models, it should be realised that the calculated values have a large uncertainty.

Modelling particle-bound deposition

Results from this thesis (**Chapter 5**) show that washing of leaves removes a large part (up to 90%) of the high MW PAHs, which most likely represents the particle-bound deposition. This suggests that, if vegetables are washed before consumption, it may be not necessary to model particle-bound deposition. However, cattle feed is not washed, and particle-bound deposition may highly contribute to the total concentration in these plants. On the other hand, when cattle feeds on pasture (or silage), soil can be ingested (~200-400 $g \cdot d^{-1}$), which may contribute to the total intake (McLachlan 1997 and references therein) and render the contribution of atmospheric particle-bound deposition less important. Nevertheless, for the uptake of SOCs in cattle, there is a need to estimate particle-bound deposition.

In the model of McLachlan (1999) the accumulation of a particle-bound compound in a plant equals the deposition minus the removal of the particle-bound compound:

$$\frac{dC_{p,pb}}{dt} = \frac{A}{V} \cdot v_{dep} \cdot C_{a,pb} - k_e \cdot C_{p,pb} \quad (6)$$

where $C_{p,pb}$ is the concentration in the vegetation due to particle-bound deposition ($mol \cdot m^{-3}$), v_{dep} is the deposition velocity or conductance of the particle-bound compound to the surface of the vegetation ($m \cdot h^{-1}$), $C_{a,pb}$ is the particle-bound concentration in the air ($mol \cdot m^{-3}$) and k_e is the first order rate constant describing weathering of the particle-bound compound from the vegetation surface (h^{-1}). Assuming that A/V, v_{dep}, k_e and $C_{p,pb}$ are constant, and integrating, the concentration in the plant is described by

$$C_{p,pb} = \frac{v_{dep} \cdot A \cdot C_{a,pb}}{V \cdot k_e} \cdot [1 - \exp(-k_e \cdot t)] \quad (7)$$

where t is time (h).

McKone and Ryan (McKone and Ryan 1989) and Lorber *et al.* (Lorber *et al.* 1994) assume that a steady state has been approached (gains equal losses, exponential term goes to zero). They formulated the model somewhat differently: instead of A/V, they incorporated $1/M_f$, where M_f represents the annual inventory of food crops per unit area (1-9 $kg.m^{-2}$, median value 3 $kg \cdot m^{-2}$; McKone).

$C_{p,pb}$ can be estimated from the K_{oa} of the compound by multiplying the gaseous concentration by the K_{oa}, and multiplying this by the particle concentration in the air ($\mu g \cdot m^{-3}$) and a constant with the value $1.22 \cdot 10^{-12}$ $m^3 \cdot \mu g^{-1}$(Finizio *et al.* 1997).

In the deposition velocity v_{dep}, dry and wet particle deposition are considered together. The deposition velocity is dependent on the particle size distribution of the compound, precipitation, the wind speed (in fact, micrometeorology in general), and the plant characteristics (McLachlan 1999). The precise relationships of these factors with the deposition velocity are unknown.

The deposition velocity can be "directly" measured from the change in plant concentration per unit ground surface divided by the concentration in the atmosphere (Jones and Duarte-Davidson 1997). There is a wide range of reported values for the deposition velocity of particles and also for particle-bound SOCs. McKone and Ryan mention studies which reported particle deposition velocities of 0.04-6500 $m \cdot h^{-1}$, and 0.1-36 $m \cdot h^{-1}$ (McKone and Ryan 1989 and references therein). Measurements on particle deposition showed that the deposition velocity to forest, grass and other plants has a minimum in the particle-size range 0.1-1 µm (Davidson *et al.* 1982, Peters and Eiden 1992), which is the relevant size range for SOCs (**Chapter 2**). Davidson reported values for the deposition velocities of this particle size ranging from ~0.4-1.0 $cm \cdot s^{-1}$, depending on the plant species (Davidson *et al.* 1982).

Although many reported data on deposition velocities for particles "alone" (i.e. without associated SOCs) are available, data for particle-bound SOCs are scarce (Douben *et al.* 1997). In most cases, deposition velocities are calculated for gaseous and particle-bound deposition as a whole. This is the case for deposition velocities of PCDD/Fs and PAHs measured by Kaupp

et al. (1994), being 0.36 $m \cdot h^{-1}$, and by Smith *et al.* (1995), who reported a deposition velocity of 28 $m \cdot h^{-1}$ for 2,3,7,8- TCDD to tall grass.

The effect of wind speed on the particle deposition velocity can be significant. Chamberlain (1967) showed an eightfold increase in the deposition velocity by an increase of wind speed from 360 to 5800 $m \cdot h^{-1}$. The nature of the receiving surface is also essential: Measurements with sticky artificial grass showed a deposition velocity which was a factor of 3 higher than for real grass (Chamberlain 1967). Additionally, in this thesis, leaf hairs have been found to increase PAH concentrations in plants with a factor of 2-5 (**Chapter 4**).

In their model for human exposure to chemicals through food chains, McKone and Ryan (1989) propose a deposition velocity of 300 $m \cdot d^{-1}$ (12.6 $m \cdot h^{-1}$). The authors mention that the uncertainty in the value is large, due to both the natural variability in the processes and the lack of complete information on the parameters (McKone and Ryan 1989).

The removal rate of particles by weathering (k_e) is a complex (and unknown) function of turbulence, precipitation, aerodynamic surface roughness and presence of leaf hairs. A recent review article on particle deposition and resuspension gives an overview of measured half-lives of particles on vegetation, mostly ranging from 9-34 days (Smith and Jones, 2000 and references therein). This means a removal rate of $8.5 \cdot 10^{-4}$-$3 \cdot 10^{-3}$ h^{-1}. McKone and Ryan (1989) assume a similar removal rate ($4 \cdot 10^{-4}$-$4 \cdot 10^{-3}$ h^{-1}, with a median of $1.25 \cdot 10^{-3}$). In radionuclide modelling studies a half-life of 14 days (removal rate of $2 \cdot 10^{-3}$ h^{-1}) is often used (Chamberlain 1970). Kinnersley (Kinnersley *et al.* 1996) measured the half lives of particles on wheat and found much lower half-lives: 1-2 days for unsheltered, and 3-4 days for sheltered plants. (k_e= 0.01-0.03 and 0.007-0.01 d^{-1}, respectively).

Conclusion particle-bound deposition

For cattle feed, the contribution of particle-bound deposition is recommended to be included in EUSES. There is a large range of reported deposition velocities of particles and particle-bound SOCs. Just as for gaseous deposition, there is a lack of knowledge on the relationships of the deposition velocity with the plant species and the environmental conditions. Calculated concentrations in plants using a “standard” deposition velocity will therefore be uncertain. The proposed value of McKone and Ryan (1989) of 12.6 $m \cdot h^{-1}$ seems a reasonable value for this “standard” deposition velocity. For the removal rate of particles, a value of $2 \cdot 10^{-3}$ h^{-1} seems plausible.

Conclusions

While in this chapter three distinct deposition mechanisms were recognised, as this is convenient for the evaluation of the predictive models, it has to be noted that for most SOCs, a combination of deposition mechanisms may occur.

For equilibrium partitioning, calculated plant concentrations are relatively similar to laboratory determined values. Difference with field-determined values are larger and possibly caused by too low values for the lipid content. The use of plant specific parameters has little advantage. As the environmental conditions have a large effect on the plant characteristics, the latter cannot be estimated precisely enough to improve the model predictions.

For non-equilibrium gaseous deposition the model predictions are uncertain. This is due to the large variation in the conductance (or deposition velocity) under influence of the environmental conditions and the plant characteristics.

For prediction of the concentration of SOCs in vegetables, it may be not necesssary to include particle-bound deposition. For cattle feed however, it is recommended that the deposition of particle-bound SOCs is included in the model. Just as for the gaseous deposition velocity, the variation in the particle-bound deposition velocities of SOCs is large, so predictions will have a high uncertainty.

Due to the high uncertainties in model predictions, caused by variation in both the environmental conditions and the plant species, the uncertainty in model predictions is high. As the relationship of plant concentrations with the environmental conditions and plant species is very complex, the accuracy of model predictions is not likely to improve much in the future.

For the screening of human health risks due to food consumption, as performed in EUSES, the uncertainty in the calculated concentrations cannot be reduced, as the model assumes “standard” environmental conditions and plant parameters. This should be realised when assessing risks with model predictions. In the case of risk assessment of incidents, input values may be chosen which are representative for the situation at hand, which will improve the precision of the calculation. However, if an accurate concentration in plants is needed for risk assessment, concentrations should be measured and not calculated with a model.

Acknowledgement

Thanks to Tjalling Jager and Johannes Lijzen from the National Instiute of Public Health and the Environment for their helpful discussions.

10

Summary AND general DISCUSSION

Summary

This thesis describes how the deposition of semivolatile organics compounds (SOCs) to plant surfaces is affected by plant properties. In the literature review (**Chapter 2**), it was concluded that differences between SOC concentrations in different plant species are often very small (< a factor of 8), although sometimes also large differences (up to a factor > 50) are measured. The plant properties that were found to cause these differences are listed (**Chapter 2**). Under equilibrium conditions, the content and the composition of plant lipids are relevant. The composition of cuticular waxes also effects the tortuosity of the cuticle, and therefore the diffusion rate of the compound into the leaf interior. For kinetically limited gaseous deposition and particle-bound deposition, the age and the architecture of the plants (surface area, aerodynamic surface roughness and orientation of the leaves) are key factors.

In the research described in **Chapter 3**, the lipid composition of the test plants *Plantago major* and *Lactuca sativa* was studied, in order to determine the possible change in composition with leaf age and the suitability of various solvents to extract cuticular waxes. The composition of the waxes was nearly independent of the age of the leaves. Extractable cuticular wax of *P. major* mainly consists of linear alkanes and triterpene acids, while wax of *L. sativa* consists of long chain alcohols and alkanes. Whereas the cuticular wax of *L. sativa* is extractable with all tested solvents, the triterpenic acids of *P. major* cannot be extracted with n-hexane.

Three *Plantago* species, namely *P. major, P. lanceolata* and *P. media,* were placed in an open greenhouse for three weeks. The PAH concentrations in these plants were compared (**Chapter 4**). The differences in PAH concentrations between the species were due to differences in plant characteristics. Since an equilibrium was probably not approached during the time of the experiment, the lower aerodynamic surface roughness, the thicker laminar boundary layer and/or higher leaf overlap of *P. media* most likely caused the lower concentrations of low MW PAHs (178 and 202) in this plant. High MW PAHs (MW 252 and 276) were most abundant in *P. media* leaves, due to the hairy surface of these leaves. When normalized to projected surface area, the concentrations of PAHs with MW 228 were also highest in *P. media*.

The distribution of the deposited PAHs over the wash-off, the extractable wax and the remaining “interior” fraction of leaves of *P. major* (young) and *P. media* (old and young) changed with MW of the PAHs (**Chapter 5**). The amount in the wash-off fraction increased with increasing MW of the compound, most likely due to larger particle-bound fractions of these compounds in the atmosphere. On the other hand, the amount determined in the interior of the leaves decreased with increasing MW of the PAHs. This is probably caused by a lower diffusion rate of higher MW PAHs.

Total PAH concentrations in old leaves of *P. media* were compared to those of old leaves of clipped *P. media* plants (**Chapter 6**). From this latter group of plants the upper leaves

were removed to create plants with a lower aerodynamic surface roughness and a lower leaf overlap. A lower aerodynamic surface roughness may lead to a lower deposition (due to less turbulence) and a lower leaf overlap to a higher deposition (due to a larger accessible leaf area). The PAH concentrations in the clipped plants were lower than those in the intact plants, indicating that aerodynamic surface roughness is more important for the deposition of PAHs than the overlapping of leaves.

P. major and *P. media* were exposed to chlorobenzenes in a flow-through system (**Chapter 7**). Despite the differences in aerodynamic surface roughness, leaf overlap and amount of leaf hairs between the different leaves, for each specific chlorobenzene the rate constants for the three groups of leaves were similar. This indicates that the dominating resistance for the uptake of chlorobenzenes in the two *Plantago* species resides in the cuticle. The chlorobenzenes are already detected in the interiors of leaves after an exposure time of 3 hours; the fraction in the leaf interior increasing with decreasing MW of the compound.

In a case study, the PAH concentrations from soil, grass and *P. major* samples were determined at different distances (0-4 km) from an oil refinery in Zelzate, Belgium (**Chapter 8**). Extremely high concentrations were found in soil samples close to the oil refinery. Also, concentrations in plant samples were high, although they were comparable to those found in other industrial areas. PAH concentrations decreased rapidly with increasing distance from the source. A shift in the PAH profile was found in the plants (although at different sites for the different species), indicating that for plants at larger distances from the source gaseous deposition of PAHs becomes more important. The ratio of the PAH concentrations in the two plant species was not constant for the different sample sites. Therefore, the concentration ratio cannot be used in predictive models.

Two predictive models, one for dry gaseous deposition (Trapp and Matthies 1995) and one for particle-bound deposition (McLachlan 1999) were evaluated (**Chapter 9)**. For dry gaseous deposition, calculated K_{pa} values were within a factor of 5-7 from laboratory derived values and within a factor 5-20 from values measured in the field. Under non-equilibrium conditions, measured elimination rate constants covered a range of several orders of magnitude, due to different plant species and environmental conditions. The elimination rate constants calculated with the model are generally at the high end of this range. It is not likely that the predictions will improve in the future, as the quantitative relationships between the environmental conditions and the plant parameters (incl. conductance) are highly complex and not known. For the consumption of vegetables by humans, particle-bound deposition of SOCs is probably not a significant process, as particle-bound compounds are largely removed from the plants by washing. For cattle feed however, it is recommended to include particle-bound deposition of SOCs into the European Union System for the Evaluation of Substances.

General discussion

Choice of plant species

P. major and *L. sativa* appeared to be highly suitable species for using them in studies on the deposition of SOCs, as their wax composition did not vary with leaf age (**Chapter 3**). Furthermore, the plants had a very different cuticular wax composition, which is relevant when studying the role of wax composition on the deposition of SOCs. However, *L. sativa* has not been used as a test species in subsequent experiments. One of the reasons for this was the shape of the plant; the outer leaves protecting the inner leaves from deposition. A second, more important reason was that *L. sativa* and *P. major* do not only differ in wax composition, but also in plant architecture. While the composition of cuticular waxes influences the equilibrium partitioning of gases, and also determines the diffusion rate of compounds through the cuticle, the plant architecture is important for kinetically limited gaseous and particle-bound deposition. As a consequence, plants with similar wax characteristics and different plant architecture were used in deposition studies, rather than plants which differed in both the wax characteristics and plant architecture. Therefore, three *Plantago* species, with similar wax characteristics but differing in plant architecture, were chosen as test plants for deposition experiments. These plants also had the additional advantage that they grow in all seasons (except at very low temperatures).

Particle-bound deposition of PAHs

The relatively high concentrations of high MW PAHs measured in *P. media* leaves are most likely caused by the effective particle scavenging of the hairy leaves of this plant (**Chapter 4**). To further investigate the importance of particle-bound deposition of PAHs and additionally, the fate of deposited PAHs, the distribution of the deposited PAHs over the wash-off, extractable cuticular wax and leaf interior fractions was studied (**Chapter 5**). The amount in the wash-off fraction increased with increasing MW of the compound. This phenomenon again points to larger particle-bound deposition of high MW PAHs. So, although air concentrations have not been measured during the semi-field experiments and the particle-bound fractions of the PAHs are therefore unknown, there is fair evidence of particle-bound deposition of PAHs to the plants from our experiments.

Several other researchers point to the relevance of particle-bound deposition of PAHs and PCDD/Fs (*e.g.* Nakajima 1995, Kaupp 1996, see **Chapter 2**). According to McLachlan's framework (McLachlan 1999), which was developed on the basis measurements of PCDDF/s in grass, particle-bound deposition is not expected to be the dominant deposition mechanism for

SOCs with $\log K_{oa}$ values smaller than 11. However, at a given K_{oa} value, particle-bound fractions of PAHs are higher than those of PCDD/Fs (Böhme 1999, Kaupp 1996). From the present study it may be concluded that particle-bound deposition is the main deposition pathway for PAHs with MW ≥ 252. For *P. media*, this pathway is also dominating for PAHs with MW 228, while for *P. major* it is not the most important pathway, but still significant (**Chapter 4, 5**).

Comparing the two semi-field experiments

The concentrations in the plants in the second semi-field experiment (field, **Chapter 5**) are higher than the concentrations in the first semi-field experiment ("open" greenhouse, **Chapter 4**). However, this comparison is not fair, as in the open greenhouse experiment the concentrations in the leaf interiors were not determined. To compare the two experiments in a just manner, the amounts in the leaf interiors (determined in the field experiment) need to be subtracted from the total concentrations in the field experiment. After subtraction, the concentrations in the field experiment are still higher than those in the open greenhouse experiment. This finding cannot be explained by the effect of the temperature, as this was higher in the second experiment than in the first. The higher concentrations in the field experiments are probably caused by the longer exposure time (3-10 weeks versus 13 days) and likely also by different exposure regimes in the greenhouse and in the field. However, as air concentrations were not determined during the experiments, neither the exposure concentrations, nor the vapour-particle distribution of the PAHs is known. Due to the lack of these data, differences between experiments cannot be adequately explained.

In the "open" greenhouse experiment of **Chapter 4** the concentration differences in cuticular wax of *Plantago* species were explained by the differences in plant characteristics. In this experiment, however, the concentrations in the leaf interiors were not measured. As is indicated in the field experiment (**Chapter 5**), the interior of the leaves of both *P. major* and *P. media* contain high fractions of low MW PAHs. This finding may have consequences for the conclusions of **Chapter 4**. Nevertheless, the fractions found in the leaf interiors are similar for the different species (~90% for MW 178, ~50% for MW 202, **Chapter 5**). Therefore, the concentration differences between the species measured in **Chapter 4** are not caused by a different distribution of PAHs over the three fractions, but by the difference in plant architecture and leaf hairs. Hence, the conclusions of **Chapter 4** remain valid.

In **Chapter 4** the plant concentrations were normalised on the projected leaf area to estimate the plant concentration per unit accessible leaf area. However, in **Chapter 6**, it apppeared that the effect of the projected leaf area was small compared to the effect of the aerodynamic surface roughness. Therefore, normalising plant concentrations on projected leaf area is not recommended.

As the hairy leaves of *P. media* can more effectively retain particle-bound PAHs than the glabrous *P. major* leaves (**Chapter 4**), it was expected that the wash-off fraction of high MW PAHs was higher for *P. media* than for *P. major* (**Chapter 5**). Although the average wash-off fraction of the old *P. media* leaves was indeed consistently higher than that of *P. major*, the differences were not statistically significant.

Plant characteristics

For high MW (particle-bound) PAHs, the presence of leaf hairs is an important plant parameter, as hairy leaves are better particle scavengers, by cushioning the impact and/or preventing particle bounce-off, blow-off and wash-off (**Chapter 4, 5**). Leaf hairs may also be of importance in dry gaseous deposition, by increasing the laminar boundary layer (**Chapter 4**). Under field conditions, the aerodynamic surface roughness plays a significant role for both gaseous and particle-bound PAHs, as the concentrations of all PAHs were higher in the intact plants than in the clipped plants (**Chapter 6**). For both low and high MW PAHs, this parameter appeared to be more significant than leaf overlap (**Chapter 6**).

Concentrations in leaf interiors

In the laboratory experiment, the chlorobenzenes were already present in the leaf interiors of *P. major* and *P. media* after three hours of exposure. This is in contrast with results from the field experiment, in which benzo[k]fluoranthene (BkF) was only found in the interior of the oldest group of leaves (8-10 weeks old; **Chapter 6**). Although the molar volume of BkF is larger than that of the chlorobenzenes (195 cm^3/mol and 121-145 cm^3/mol, respectively, calculated with McGowan's characteristic volumes (Abraham and McGowan 1987), which leads to a lower diffusion coefficient for BkF, the difference between weeks and hours is very large. However, the short time needed for crossing the extractable wax is in agreement with transfer times estimated with diffusion coefficients in cuticular waxes (both epi- and intracuticular waxes) of other plant species. For molar volumes of 121-195 cm^3/mol, the diffusion coefficients in waxes are in the order of 10^{-16}-10^{-17} $m^2 \cdot s^{-1}$ (extrapolated from values obtained for *Hordeum vulgare, Picea abies* and *Fagus sylvatica* Schreiber and Schönherr 1993, Schreiber 1995, Schreiber *et al.* 1996). The amount of extractable cuticular wax per cm^2 of *Plantago* leaves is ~0.03 mg. Assuming a wax density of 1 $kg \cdot L^{-1}$ (Baur *et al.* 1999) yields a volume of 30 $\mu m^3 \cdot cm^{-2}$ of leaf. The thickness of the wax layer is therefore ~0.3 µm. Using Fick's Law, it can be calculated that the minimum time needed to diffuse through the extractable wax layer is 4-44 minutes.

So BkF needs more time to reach the interior compared to the estimated 4-44 minutes due to the low tendency of this very hydrophobic compounds to desorb from the particle and partition to the cuticle. Another possibility is that the dryer field conditions in the field have induced thicker cuticles

(Cape and Percy 1993), which may have led to longer transfer times. However, the extractable cuticular wax contents of the plants in both experiments were similar. Hence, this explanation is not very plausible.

For both the field experiments and the laboratory experiments the fraction found in the leaf interiors is decreasing with increasing MW of the compound. So, although the compounds can reach the interiors of the leaves rapidly (as in the laboratory experiment), the transfer rate is determined by the diffusion in the extractable waxes.

Concentration ratios

In the case study (**Chapter 8**), the ratio of the PAH concentrations in the *Plantago major* and grass (mixed species) was not constant for the different sample sites. Therefore, the concentration ratio cannot be used in predictive models. To test the principle of the constancy of the concentration ratios, the concentration ratios for the *Plantago* species in the semi-field experiments were calculated.

In the open greenhouse experiment (**Chapter 4**), the concentration ratios of the total PAH concentrations in the three *Plantago* species are fairly constant for samples from Day 6, 13 and 20 (*P. major*/*P. lanceolata* 1.4 ± 0.2, *P. major*/*P. media* 2.6 ± 0.4, *P. lanceolata*/*P. media* 1.8 ± 0.2). In this experiment, in contrast with the case study in Zelzate, the leaves of the three species were exposed for equal time periods, leading to reproducible concentration ratios. However, on Day 0 of the greenhouse experiment (**Chapter 4**) the ratios are different (2.8 ± 0.6, 7.8 ± 4 and 2.8 ± 1.5, respectively), probably because the exposure regime until Day 0 (closed greenhouse) was different from that of Day 1 to Day 20 (open greenhouse).

The total PAH concentration ratio *P. major*/*P. media* from the field experiment (**Chapter 5**) is 0.3 ± 0.2. This is a difference of a factor of 10 from the value of 2.6 ± 0.4 in the open greenhouse experiment. This is explained by the different exposure regime (and also by the fact that the groups of leaves have different ages).

So, while in “controlled” experiments with a fairly constant exposure regime the concentration ratios are similar, the concentration ratios of plants which experienced different exposure times and regimes are not constant.

Conclusions

- Dry gaseous deposition is the main deposition pathway to *Plantago* plants for PAHs with low MW (MW $\leq$ 202). For high MW PAHs (MW $\geq$ 228) particle-bound deposition is significant.

- Leaf hairs enhance the deposition of particle-bound PAHs on the leaf surface. Washing of leaves effectively removes particle-bound PAHs.

- PAH concentrations in plants increase with increasing aerodynamic surface roughness of the plant. The aerodynamic surface roughness has a larger effect on the deposition of PAHs to the investigated *Plantago* plants than the overlapping of leaves.

- Transport of SOCs through the extractable cuticular wax of *Plantago* is important for low MW PAHs and chlorobenzenes. The fraction of compounds present in the leaf interior decreases with increasing MW of the compound.

- Differences in plant characteristics between *Plantago* species lead to differences in PAH concentrations, but the concentration differences are not very large (< factor of about 10).

- Plants which experienced different exposure regimes and times do not have constant concentration ratios. Therefore, concentration ratios cannot be used for an accurate prediction of PAH concentrations in plants.

- Current models can only roughly predict SOC-concentrations of plants in the field (especially under non-equilibrium conditions) due to the high variation in environmental conditions and plant characteristics.

- For the prediction of the concentration of SOCs in vegetables, it may not be necessary to include particle-bound deposition. For cattle feed, however, it is recommended that the deposition of particle-bound SOCs is included in models such as EUSES.

- For “continuous” emissions, the use of plant specific parameters in predictive models has little advantage, because (1) plant parameters are not constant, but dependent on environmental conditions and (2) environmental conditions also have a large, but quantitatively unknown effect on the SOC-concentrations of the plants. In case of incidents, the use of values for specific plants may improve the precision of the predictions. However, if accurate concentrations in plants are needed for risk assessment, concentrations should be measured and not calculated with a model.

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Samenvatting

Kader van het onderzoek

De lucht, vooral boven steden en industriegebieden, is vervuild met veel verschillende chemische stoffen. Een aantal van deze stoffen kan worden opgenomen door planten. Dit geldt vooral voor semi- (= half-)vluchtige organische stoffen (SOS), omdat deze net vluchtig genoeg zijn om in de lucht voor te komen, maar aan de andere kant niet zó vluchtig zijn dat ze vanuit de plant direct weer naar de lucht ontsnappen. Voorbeelden van SOS zijn polycyclische aromatische koolwaterstoffen (PAKs), chloorbenzenen en polychloorbifenylen (PCBs). Dit zijn tevens de stoffen die in dit onderzoek zijn bestudeerd.

PAKs ontstaan bij verbrandingsprocessen (o.a. bosbranden, automotoren, verwarming) en komen in relatief hoge concentraties voor in de lucht. Ze bestaan uit gefuseerde benzeen-ringen. De in dit proefschrift bestudeerde PAKs hebben drie tot zes ringen. Chloorbenzenen ontstaan vaak als bijprodukten bij het maken van chloorhoudende chemische stoffen. PCBs zijn in het verleden gebruikt in elektrische installaties en in hydraulische systemen. Vanaf de jaren zeventig is de toepassing ervan in veel geïndustrialiseerde landen verboden. Door lekkages uit transformatoren e.d. en door stort van PCB-houdende apparatuur komen deze stoffen echter nog altijd in het milieu terecht.

Sommige SOS zijn acuut giftig, maar het gevaar van deze stoffen in het milieu wordt vooral veroorzaakt door langdurige blootstelling aan relatief lage concentraties. Bovendien is een aantal van deze stoffen (bijv. een aantal PAKs) kankerverwekkend. De SOS hebben met elkaar gemeen dat ze lipofiel (letterlijk: vetminnend) zijn. De meeste SOS zijn persistent, dat wil zeggen dat ze slechts zeer langzaam in het milieu worden afgebroken. Zo zitten we nu nog met de PCBs opgescheept die dertig tot veertig jaar geleden in het milieu zijn gebracht.

In de lucht komen SOS voor als gassen, maar ze zijn ook aanwezig gebonden aan minuscule stofdeeltjes (of aerosolen). Beide “vormen” kunnen vanuit de lucht in/op de plant terechtkomen: dit wordt atmosferische depositie genoemd.

De buitenkant van de plant, de cuticula, is een vette, wasachtige laag die de plant tegen uitdroging beschermt. De cuticula bestaat uit bladwas en cutine (een soort netwerk van koolstofketens). Bij de depositie van gasvormige stoffen treedt er een verdeling op tussen de cuticula en de lucht; de stoffen lossen er als het ware in op. Hoe enorm vetminnend SOS zijn blijkt uit de sterke ophoping in de plant: Als er evenwicht tussen lucht en plant wordt bereikt kan de concentratie in de plant 100.000 tot 100 miljoen keer groter zijn dan de concentratie in de lucht. Na opgenomen te zijn, kan een stof door de cuticlula verder de plant binnendringen.

De depositie van stoffen die aan deeltjes gebonden zijn, is in feite niets anders dan het “landen” van de deeltjes op het bladoppervlak.

De depositie van deze stoffen in/op planten geeft aanleiding tot bezorgdheid, want als planten worden gegeten, komen de stoffen in dieren en mensen terecht. Mensen krijgen door het eten van vooral vlees, zuivelprodukten en groenten veel meer SOS binnen dan met het inademen van de lucht. Om de risico's van deze stoffen voor de mens te schatten, wordt met modellen geprobeerd te voorspellen wat de concentratie in de planten zal zijn, bij een gegeven concentratie in de lucht. Een vraag daarbij is, of er in deze modellen rekening moet worden gehouden met de eigenschappen van de plant, zoals bijvoorbeeld de architectuur (d.w.z. de grootte van de plant, het aantal, de grootte en de positie van de bladeren, het totale bladoppervlak, etc.) en de aanwezigheid van haren op het blad.

Inhoud proefschrift

In het onderzoek is bestudeerd welke planteigenschappen een effect hebben op de atmosferische depositie van SOS. In **hoofdstuk 2** is een overzicht gegeven van de wetenschappelijke literatuur. Van een tweetal testplanten is de bladwas nader onderzocht (**hoofdstuk 3**). Planten met verschillende eigenschappen zijn vervolgens blootgesteld aan SOS (in het veld - **hoofdstuk 4-6** en **8** of in het lab - **hoofdstuk 7**). De stoffen werden uit de planten gehaald door extractie met een oplosmiddel. Na opzuivering van de monsters werd de hoeveelheid in de extracten gemeten. Tenslotte is beschreven of de concentraties van stoffen in planten met modellen kan worden voorspeld, in plaats van gemeten (**hoofdstuk 9)**.

In het literatuuroverzicht (**hoofdstuk 2**) wordt geconcludeerd dat de concentraties van SOS in verschillende planten meestal niet zo sterk verschillen (meestal minder dan een factor 8). Soms zijn echter wel grote verschillen (groter dan een factor 50) te vinden. De planteigenschappen die voor de verschillen verantwoordelijk zijn, zijn het gehalte en de samenstelling van lipiden (vetten), de leeftijd en de architectuur van de plant.

In **hoofdstuk 3** wordt de samenstelling van de bladwas van twee planten (grote weegbree en sla) beschreven, en hoe deze verandert met de leeftijd van de plant. Bovendien is nagegaan welke oplosmiddelen gebruikt kunnen worden voor het extraheren van bladwassen. De wassamenstelling bleek nauwelijks te veranderen met de leeftijd. De bladwas van sla kan worden geëxtraheerd met alle gebruikte oplosmiddelen, maar het oplosmiddel hexaan bleek ongeschikt voor de extractie van bladwas uit weegbree.

In **hoofdstuk 4** is een experiment beschreven waarin verschillende soorten weegbree in een "open kas" (d.w.z. een kas met een dak en halve wanden) in de Botanische Tuinen werden gezet. De gebruikte soorten zijn grote weegbree (*Plantago major*, een relatief grote plant met grote, gladde bladeren die vrij in de lucht staan), smalle weegbree (*Plantago lanceolata*, eveneens relatief groot, met smalle, gladde bladeren) en ruige weegbree (*Plantago media*, een lage plant met kleine, behaarde bladeren die elkaar gedeeltelijk overlappen). De gebruikte planten verschillen dus sterk in architectuur, maar hun bladwas-eigenschappen zijn ongeveer

gelijk. Na een aantal weken blootstelling aan de buitenlucht zijn de hoeveelheden PAKs in de bladeren gemeten. Hieruit bleek dat weegbree met behaarde bladeren (*P. media*) meer deeltjesgebonden stoffen invangt dan weegbree met gladde bladeren (*P. major* en *P. lanceolata*), terwijl het voor de gasvormige stoffen juist andersom is. Voor de deeltjesgebonden stoffen kan dit verschil worden verklaard door aan te nemen dat de haren op het blad ervoor zorgen dat deeltjes beter op het blad blijven zitten. Voor de hogere concentratie gasvormige PAKs in de planten met gladde bladeren worden drie verklaringen gegeven. Ten eerste kan de grotere hoogte van *P. major* en *P. lanceolata* hebben gezorgd voor meer turbulentie in de lucht direct rondom de plant en daarmee voor een snellere aanvoer van PAKs. De tweede verklaring is dat het harendek van *P. media* een dikkere laag stilstaande lucht veroorzaakt rond de bladeren van deze plant, waardoor het langer duurt dat de stoffen door deze laag heen zijn getransporteerd. Ten derde kan het feit dat bij *P. media* de bladeren elkaar sterk overlappen en zo elkaar afschermen, een oorzaak zijn voor de lagere concentraties PAKs.

De plaats waar PAKs zich bevinden nadat ze op de plant zijn terechtgekomen, is onderzocht in **hoofdstuk 5**. Planten (*P. major* en *P. media*) werden op een proefveld in de Botanische Tuinen uitgezet. Na de oogst werden de bladeren werden gespoeld met een waterige oplossing om de PAKs die zich op het bladoppervlak bevonden te verwijderen. Daarna werden de bladeren gedompeld in een oplosmiddel zodat de bladwassen (met daarin PAKs) werden opgelost. Het restant van de bladeren werd vermalen. In de drie zo verkregen fracties (afspoel-, bladwas- en interne fractie) werden de hoeveelheden PAKs bepaald. Uit de resultaten bleek dat de gasvormige stoffen in de cuticula worden opgeslagen, maar dat de stoffen die aan deeltjes gebonden zijn veelal op het bladoppervlak blijven liggen. Deze laatste blijken ook weer gemakkelijk van het blad te worden afgespoeld. Het wassen van groenten is dus zeker zinvol!

Het effect van de grootte van de plant en het overlappen van de bladeren op de hoeveelheid ingevangen stoffen is nader bestudeerd in **hoofdstuk 6**. De PAK-concentraties in oude bladeren van intacte ruige weegbreeplanten werden vergeleken met de concentraties in oude bladeren van planten waarvan de top was weggeknipt. Het knippen had twee effecten: (1) de geknipte planten waren kleiner (lager) dan de intacte, en (2) de (overgebleven) bladeren overlapten elkaar minder dan bij de intacte planten. Het effect van (1) is dat lagere planten wellicht minder PAKs invangen dan hogere, door de lagere turbulentie die ze veroorzaken. Het effect van (2) is dat minder bladoverlap zou kunnen leiden tot meer PAKs per m^2 blad, omdat per blad meer bladoppervlak direct is blootgesteld aan de lucht. De PAK-concentraties in de intacte planten bleken hoger dan die in de geknipte planten. Hieruit blijkt dat de hoogte van de ruige weegbree belangrijker is voor de invangcapaciteit van PAKs dan de mate van bladoverlap.

P. major en *P. media* werden blootgesteld aan chloorbenzenen in een blootstellingskast in het laboratorium (**hoofdstuk 7**). Er werd nagegaan hoe snel de stoffen werden opgenomen in de beide planten en hoe snel ze in de bladwas en in het binnenste van het blad waren te vinden. Voor de verschillende planten waren de opnamesnelheden voor elke chloorbenzeen ongeveer

gelijk. Dit is een aanwijzing dat de traagste stap in het opnameproces voor de chloorbenzenen de opname in de cuticula is en niet de aanvoer door de lucht. Als de aanvoer door de lucht de traagste stap zou zijn, dan zouden er namelijk verschillende opnamesnelheden voor behaarde en onbehaarde planten of grote en kleine planten zijn gevonden. Chloorbenzenen konden al na drie uur blootstelling in het binnenste van het blad worden aangetoond. De relatieve hoeveelheid in het binnenste neemt af met de grootte van het molekuul.

In dit onderzoek is één casus onderzocht (**hoofdstuk 8**). Het betrof een vervuiling in Zelzate (Vlaanderen), waar een olieraffinaderij in het centrum staat. De PAK-concentraties in grond, gras en grote weegbree werden bepaald op verschillende afstanden (0-4 km) van de raffinaderij. Dichtbij de fabriek werden in de grond uitzonderlijk hoge concentraties gemeten. De concentraties in de planten waren ook erg hoog, maar deze kwamen min of meer overeen met concentraties die in andere industriegebieden worden gemeten. De concentraties daalden snel met toenemende afstand tot de fabriek. Op grotere afstand van de bron werden er relatief minder deeltjesgebonden PAKs in de planten gemeten, maar in de grond bleef de verhouding gasvormige/deeltjesgebonden PAKs gelijk. Dit wijst erop dat voor planten depositie van gassen relatief belangrijker is dan depositie van deeltjesgebonden stoffen.

Zoals eerder beschreven, kunnen SOS giftig zijn voor de mens. Bij de risicoschatting wordt getracht de hoeveelheden van de stoffen in de plant met modellen te berekenen met behulp van gemeten of geschatte concentraties in de lucht (**hoofdstuk 9**). Uit bovengenoemde hoofdstukken blijkt dat de architectuur en de beharing van de plant de depositie beïnvloeden. Het gebruiken van plant-specifieke waarden in de modellen om de concentraties in de plant beter te kunnen schatten, heeft echter niet veel voordelen. Behalve de eigenschappen van de plant zijn namelijk ook de milieu-omstandigheden, vooral temperatuur en wind, belangrijk voor de depositie op de plant. Omdat de relaties tussen de temperatuur/wind en de opnamesnelheden vooralsnog onbekend zijn en milieu-omstandigheden bovendien zeer variabel zijn, kunnen modellen nooit heel precies berekenen hoeveel stoffen er in de plant zitten. Om nauwkeurig (d.w.z. binnen een factor ~10) te weten in welke concentraties de stoffen aanwezig zijn in een plant, zullen deze moeten worden gemeten in plaats van berekend.

Bij de risicobeoordeling van SOS is op dit moment de deeltjesgebonden depositie op planten niet in de voorspellende modellen opgenomen. Toch levert dit proces voor de zeer lipofiele SOS een grote bijdrage aan de totale hoeveelheid SOS in planten. Omdat groenten worden gewassen voor consumptie, waardoor de deeltjesgebonden SOS grotendeels weer worden afgespoeld, is voor mensen weinig extra risico van de depositie van deeltjesgebonden SOS te verwachten. Planten die door vee worden gegeten worden echter niet gewassen. Daarom wordt aanbevolen de deeltjesdepositie naar plantaardig veevoer aan de modellen toe te voegen.

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Curriculum vitae

Op 5 juli 1970 werd ik (Martine Bakker) geboren in Rotterdam. Achttien jaar later behaalde ik mijn VWO-diploma op het Revius Lyceum in Doorn. Ondanks (of misschien juist omdat) het laagste cijfer op mijn eindlijst voor scheikunde was, begon ik een paar maanden later aan de studie scheikunde in Utrecht. In het derde jaar werd de overstap naar de kopstudie milieukunde gemaakt. Het keuzevak bij milieukunde, uitgevoerd samen met Else Sneller, betrof onderzoek naar de opname van hexachloorbenzeen door gerst, bij de projektgroep Transportfysiologie (Prof. C. Kollöffel) aan de faculteit Biologie. Mijn afstudeeronderzoek deed ik bij de vakgroep Natuurwetenschap & Samenleving van de faculteit Scheikunde (prof. dr. H.A.M. de Kruijf). Het praktisch gedeelte hiervan werd uitgevoerd bij de Milieuchemie-groep van het Research Instituut voor Toxicologie (RITOX, prof. W. Seinen), waar ik onder begeleiding van Marca Schrap het sorptiegedrag van chloorbenzenen en PAKs bestudeerde. Toen bleek dat ik dit onderzoek ook voor de studie scheikunde kon laten tellen, werd ik weer aankomend scheikundige. Na het inhalen van een zestal tentamens en een practicum ontving ik in mei 1994 mijn doctoraaldiploma scheikunde. Er bleek nog precies genoeg tijd over voor een afstudeeronderzoek voor de studie milieukunde. Dat resulteerde in een stage bij het milieuadviesburo Chemielinco in Utrecht, waar ik me heb verdiept in het inschatten van bodemverontreiniging aan de hand van historische informatie. In augustus 1994 kreeg ik het diploma milieukunde uitgereikt. Vervolgens was ik van 1 november 1994 tot 1 augustus 2000 voor 80% van de werkweek aangesteld als onderzoeker in opleiding bij de faculteit Biologie en werkte ik bij de eerdergenoemde groepen Transportfysiologie en Milieuchemie aan het in dit boekje beschreven onderzoek.