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Novel Diagnostic Tools to Detect and Monitor Persistent Organic Pollutants in Remote Mountainous Areas

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Abstract

In the present thesis, persistent organic compounds that belong to the families of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F), polychlorinated biphenyls (PCB), polynuclear aromatic hydrocarbons (PAH) and some organochlorine pesticides (OCP) are monitored in forested mountain areas by means of active and passive methods. Semi permeable membrane devices (SPMD) and coated stir bars (CSB) for aerial passive sampling are compared with the classical methods of active sampling and collection of needles. Quantitative determinations of the pollutants are performed using high resolution gas chromatography coupled with a high resolution mass spectrometer (HRGC/HRMS). The resulting analytical values are reinforced and complemented by applying two bioassays. The micro-EROD bioassay and a genetically modified yeast-based bioassay are carried out to determine aryl hydrocarbon receptor inducers and endocrine disrupters, respectively.

This study revealed the suitability of semi permeable membrane devices and coated stir bars as passive air samplers in mountainous regions for qualitative and semi-quantitative determinations of persistent organic pollutants. It is also shown that the results obtained by the passive sampling methods depend on several parameters such as the physicochemical properties of the compounds of interest, the time of exposure of the passive samplers and the seasonal period at which the sampling is performed. This shows that geographical factors such as altitude and mean seasonal temperatures also have to be considered when evaluating the passive sampler performance.

The uptake behaviour of SPMD and coated stir bars after ½ year exposure depended strongly on the pollutant. For OCP and PAH, the uptake in SPMD and coated stir bars correlated well, the correlation for PAH being linear also with the air concentrations. On the other hand, PCB accumulated in coated stir bars correlated well with air concentrations, but not so good with SPMD. Regarding PCDD/F, tetra- and pentachlorinated PCDD/F presented a good uptake in SPMD, whereas higher chlorinated homologues are hardly detectable or not detected (like OCDF) in these devices. This behaviour is related to the different gas-particle compound distributions and concentrations of PCDD/F in the air, where low air concentrations and low compound availability in the gaseous phase determine a poor uptake in SPMD.
In contrast, in the same exposure period, needles collected compounds in all the studied PCDD/F homologue groups and showed similar distributions at all sampling sites.

Furthermore, the distribution patterns of PAH accumulated in needles also remained without significant changes at all sampling sites and even in the different periods of collection.

The uptake behaviour of SPMD depends on the deployment time, as these devices pass from an initial linear uptake phase to a curvilinear phase until reaching an equilibrium phase. Longer exposures therefore generally implied higher compound uptake in SPMD, but compounds with intermediate to low octanol-air partition coefficient were in equilibrium or approaching equilibrium after ½ year deployment. Lower temperatures, generated seasonally or by altitudinal conditions cause higher compound accumulation.

Accumulation also depended on the altitude for some of the pollutants. Humus soil and SPMD exhibited higher accumulations at the higher sites of the altitude profiles for OCP, whereas concentrations of PAH in SPMD decreased with altitude and were not detected at all at the higher altitude sites. In contrast, needles seem to be strongly influenced by local characteristics, so no profile tendency was found for the analysed compounds.

The analytical results were complemented by applying two bioassays. Micro-EROD bioassay results were as a general trend higher than analytical results. However, samples with the highest analytical PCB values presented a lower bioassay activity (lower EROD response), which is possibly related to inhibitory effects. Higher concentrations of non-ortho PCB may be the reason for the slightly antagonistic response in the EROD bioassay. The yeast modified bioassay to determine estrogenic disrupters was successfully implemented as part of the present study and later applied to environmental samples from remote forested sites. Concentration levels of estrogenic disrupters were low in the studied areas.

To summarize, the novel passive techniques are promising tools to determine persistent organic compounds in remote areas. The bioassay determinations were complementary to the analytical methods and allowed a better data interpretation than the sole use of the analytical results.

List of Publications

I. **Levy W**, Henkelmann B, Pfister G, Kirchner M, Jakobi G, Niklaus A, Kotalik J, Bernhöft S, Schramm K-W. 2006. Comparison of PAH concentrations in Semipermeable Membrane Devices, low volume active sampler, and spruce needles. *Organohalogen Compounds*, **64**: 45-48.

II. **Levy W**, Henkelmann B, Pfister G, Kirchner M, Jakobi G, Niklaus A, Kotalik J, Bernhöft S, Fischer N, Schramm K-W. 2007. Monitoring of PCDD/Fs in a mountain forest by means of active and passive sampling. *Environmental Research*, **105**: 300-306.

III. **Levy W**, Henkelmann B, Pfister G, Bernhöft S, Niklaus A, Kirchner M, Jakobi G, Bassan R, Belis C, Jakl T, Kräuchi N, Magnani T, Perthen-Palmisano B, Schröder P, Schrott H, Sedivy I, Simončič P, Vannini P, Vilhar U, Schramm K-W. 2007. Semipermeable membrane devices (SPMD) as passive samplers: Data interpretation regarding exposure time. *Organohalogen Compounds*, **69**: 599-603.

IV. **Levy W**, Henkelmann B, Pfister G, Bernhöft S, Niklaus A, Kirchner M, Jakobi G, Bassan R, Belis C, Jakl T, Kräuchi N, Magnani T, Perthen-Palmisano B, Schröder P, Schrott H, Sedivy I, Simončič P, Vannini P, Vilhar U, Schramm K-W. 2007. Dioxin-like compounds in soils: Comparison of analytical measurements and bioassay determinations. Proceedings of SECOTOX: *Environmental Management, Engineering, Planning and Economics*, **1**: 528-532.

V. **Levy W**, Henkelmann B, Pfister G, Bernhöft S, Niklaus A, Kirchner M, Jakobi G, Bassan R, Belis C, Kräuchi N, Moche W, Offenthaler I, Simončič P, Uhl M, Weiss P, Schramm K-W. Long term studies with semipermeable membrane devices (SPMD) in montanious areas *(submitted)*.

VI. Fried K W, Bazzi R, **Levy Lopez W**, Corsten C, Schramm K-W, Bell D R, Rozman K K. 2007. Relationship between aryl hydrocarbon receptor-affinity and the induction of EROD activity by 2,3,7,8-tetrachlorinated phenothiazine and derivatives. *Toxicology and Applied Pharmacology*, **224**: 147-155.

Abbreviations and Acronyms

17α-E	17α-Ethynylestradiol
17β-E	17β-Estradiol
A	Sampler area
AhR	Aryl hydrocarbon Receptor
Arnt	Aryl receptor nuclear tanslocator
ASE	Accelerated Solvent Extraction
B	Boubin
BCF	Bio concentration factor
C	Compound concentration
C_{air}	Compound concentration in air
C_s	Compound concentration in SPMD
CSB	Coated Stir Bars
δ	Effective thickness
DDT	Dichloro Diphenyl Trichloroethane
DES	Diethylstilbestrol
EC_{50}	Effect Concentration 50 %
ER	Estrogen Receptor
EROD	7-Ethoxyresorufin-*O*-deethylase
f	Fugacity
F	Flux
H	Haidel
hERα	Human estrogen receptor α
HRGC	High Resolution Gas Chromatography
HRMS	High Resolution Mass Spectrometry
k_e	Elimination rate constant
K_H	Henry´s law constant
K_m	Membrane resistance coefficient
K_o	Total mass transfer coefficient
K_{oa}	Octanol-air partition coefficient
K_p	Particle-gas partition coefficient
K_{sa}	SPMD-air partition coefficient
K_T	Overall uptake rate constant
k_u	Uptake rate constant
LDPE	Low Density Polyethylene
LOD	Limit of Detection
LOQ	Limit of Quantification
LowVol	Low Volume
M	Mitterfels
MW	Molecular Weight
N	Normal conditions (1 atm, 273 K)
NE	North-East
NW	North-West
OCP	Organochlorine Pesticides
OCDF	Octachlorodibenzo furan
Φ	Particulate-phase fraction
PAH	Polynuclear Aromatic Hydrocarbons
PAS	Passive air samplers
PC	Principal Component
PCA	Principal Component Analysis
PCB	Polychlorinated Biphenyls
PCDD	Polychlorinated Dibenzo-*p*-dioxins
PCDF	Polychlorinated Dibenzofurans
PDMS	Polydimethylsiloxane
POP	Persistent Organic Pollutants
PRC	Performance Reference Compounds
R	Ruckowitzschachten
R^2	Correlation coefficient
R_s	Sampling rate
SE	South-East
S_o	Octanol solubility
SPE	Solid Phase Extraction
SPMD	Semi Permeable Membrane Devices
SPME	Solid Phase Micro Extraction
SW	South-West
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalent
TSP	Total Suspended Particulate concentration
TWA	Time weighted average
UPGMA	Unweighted Par Group method with Mathematical Averages
V	Sampler volume
WHO	World Health Organization
yEGFP	enhanced green fluorescent protein
Z	Fugacity capacity

1. INTRODUCTION

In the process of industrialisation, the efforts to improve the quality of life went hand in hand with technological progress, where the chemical industry has always played an important role. Surrounded by man-made compounds in every variety and quantity, the awareness of their possible harmfulness to nature and mankind only developed late, usually after experiencing severe accidents that awoke public consciousness about the way we influence the environment. Incidents in the course of the last decades such as human intoxications by polychlorinated biphenyls in food in Japan in 1968, by dioxin environmental exposure in Missouri in 1971, by an outburst of dioxins in Seveso, Italy in 1976, are just a few examples out of hundreds. These accidents were not the first ones, but have made public the potential toxicity and adverse effects of synthetic chemicals on the environment and human health. In this way, community concern, public pressure and government responsiveness started to arise in the sixties and seventies.

In particular, industrial production and wide spread use of persistent organic pollutants (POP) led to long lasting human exposure. Among them, polychlorinated biphenyls (PCB) are technical mixtures that were widely used in the industry and nowadays are still used in capacitors, transformers and welding implements because of their inherent properties as good isolators, their thermo stability and low reactivity. Another important group to mention are the organochlorine pesticides (OCP), which were used for many decades and left after-effects that are still present nowadays. Other families of compounds such as polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are produced as secondary industrial products, e.g. by chlorine bleaching, or indeliberately e.g. by incineration processes. Polynuclear aromatic hydrocarbons (PAH) are compounds that naturally occur in the environment, but the increase in industrialization raised their air levels significantly.

Persistent organic pollutants are prone to long-range transport in the atmosphere due to their persistence and volatility, such that they spread to and contaminate areas where they were never used. They travel worldwide by mechanisms of cold condensation followed by re-volatilisation, the atmosphere acting as a distillation system where the compounds condensate at different temperatures depending on their physicochemical properties and on environmental conditions (Lohmann *et al*., 2007). The fact that POP can be integrated in a new ecosystem after undertaking long-range transport generates an impact on the new environment that it is necessary to address (Wania, 2003). POP are transferred from the atmosphere to the soil

compartment by dry and wet deposition where the vegetation is the intermediate compartment between them (Smith and Jones, 2000). In this way the accumulation of lipophilic compounds in needles allows them to be used as a natural passive sampler. This accumulation is strongly related to the species (Böhme *at al*., 1999). Spruce needles (*Picea abies (L.) Karst*) are suitable to be used as natural passive sampler (Umlauf and McLachlan, 1994). However, considerations as sampling season and location as well as age of the needles can not be disregarded. Spruce needles are used in this study as natural samplers to monitor and qualitatively determine POP.

The transport mechanism favours the accumulation of POP in cold areas such as polar and mountainous regions, where the re-volatilisation decreases. In high altitude areas, the mountainous barriers oblige the air masses to ascend, decreasing their temperature and leading to condensation and POP accumulation. Therefore, mountainous areas are particularly well suited for monitoring contamination of the environment by POP. However, the difficulty to access these remote areas implies the use of novel sampling techniques to achieve an accurate knowledge of the POP deposition and adequate POP monitoring in these regions.

Classical techniques based on active volume sampling, implying energy supply, are not always logistically possible. The active volume sampler is composed of a cartridge with a glass fibber filter (to collect compounds attached to particles) and a resin (to retain gas phase compounds) connected to a pumping system. This is the most common sampling technique despite the need of energy supply and regularly maintenance. These requirements generate practical restrictions on the number and locations of the geographical sites suitable for active sampling as well as on the frequency of the monitoring.

Thus, passive air samplers (PAS), uptaking compounds by passive mass transfer, based on the free flow of compounds from the air to an accumulation matrix, are seen as an alternative to the active volume samplers (Harner *et al*., 2006). Passive samplers are cheaper in implementation and manipulation and also of easier in maintenance than the active samplers.

The potential of semi permeable membrane devices (SPMD) as passive air samplers was demonstrated in the laboratory by Petty and collaborators in 1993 (Petty *et al*., 1993). The usefulness of SPMD as aerial samplers was later confirmed in aerial determinations of OCP in the field (Prest *et al*., 1995). Advantages such as no need of energy supply and the ability of

targeting a broad range of compounds are determinant factors to choose this kind of passive sampling technology (Ockenden *et al*., 1998). Subsequently, studies to determine the presence of contaminants, their sources and their spatial distributions in air with SPMD were emerging (Ockenden *et al*., 2001; Lohmann *et al*., 2001; Meijer *et al*., 2003) and with them some important issues to be addressed: for example, the determination of the best deployment conditions, the optimal deployment period, knowledge of the SPMD uptake kinetics at the moment of sampling and the conversion from the passive sampler concentrations to the real air concentrations (Lohmann *et al*., 2001; Söderström and Bergqvist, 2004; Bartkow *et al*., 2005).

In recent years, the use of other passive samplers, e.g. coated stir bars is seen as a promising tool for passive air sampling, as well (Henkelmann *et al*., 2007). These single-phase devices consist of a sorption polymer coating a magnetic rod encapsulated in glass. The device's qualities such as low cost, possibility of re-utilization and the possibility of direct thermal desorption avoiding clean-up procedures make coated stir bars a good candidate for passive air sampling (Baltussen *et al*., 1999; Rusina *et al*., 2007).

In the present thesis, POP concentration levels are studied in European mountain forest regions, where the accumulation of POP is favoured. For this study, classic and novel techniques for air sampling are used in order to determine their respective advantages and disadvantages. The classical methods are active volume sampling employed to determine air concentrations quantitatively and spruce needle collection, utilized as a qualitative monitor screening. These sampling techniques were compared with the recent, promising passive air samplers, SPMD and coated stir bars. To reinforce the monitoring in the forested areas, soil samples acting as a final sink and providing the historical fingerprint related to POP deposition at these mountainous sites were analysed.

In the work, POP were determined analytically. Thus, each compound was analysed and quantified separately. However, the mixture of synthetic and natural organic compounds accumulated in the environment to which the living organisms are exposed can generate synergic, agonist or antagonist effects. These effects are not considered and even ignored by the single analytical determinations. Further, some of the persistent organic compounds penetrate the cell membrane due to their lipophilicity and persistence and are able to bioaccumulate leading to magnified accumulation along the trophic chain. Organic

compounds, such as aromatic hydrocarbons, are able to pass through the membrane and act as enzyme inducers through specific binding to two important receptor groups: the aryl hydrocarbon receptor (AhR) and the estrogen receptor (ER). The AhR is known to participate in multiple mechanisms of the normal physiology in vertebrates, such as reproduction, immunity and cell proliferation (Fujii-Kuriyama *et al*., 2007). It is also involved in the elimination mechanisms of xenobiotics and its activation can cause effects such as teratogenesis and tumour promotion (Hankinson, 1995; Estabrook, 1996). The disruption of endocrine systems via the ER leads to hormone-dependent diseases and to carcinogenic effects (Toppari *et al*., 1996).

As the classical analytical determinations are not able to cover these environmental aspects, two bioassays considering the potential of the sample as AhR inducer and ER disrupter are performed in this study as complementary methods: the 7-Ethoxyresorufin-*O*-deethylase (EROD) bioassay to determine the AhR inducers and a genetically modified yeast-based bioassay to determine ER disrupters. The samples from the accumulation matrices SPMD, needles and soil are used for these determinations.

The present thesis is organized as follows: In chapter 2, methods to sample and quantify POP are described as well as the theory behind them. A brief review of the studied POP is also included. In chapter 3, the results are shown and discussed using different passive sampling techniques after analytical measurements (Papers I and II). The relationships between passive and active sampling are also considered (Papers I, II and unpublished data). To complement this, the uptake behaviour of SPMD regarding time of exposure and altitude gradient of the sampling sites is analysed (Papers III and V). In a second step, the results of analytical and EROD determinations are compared (Paper IV). The EROD bioassay is applied as a routine assay (Paper VI) and the yeast-based bioassay was implemented successfully at the laboratory (unpublished data). Additionally, both bio-analytical determinations are presented together as a final data analysis of the monitored area (unpublished data). General conclusions are given in chapter 4 and finally, further investigation is proposed in chapter 5.

This work forms part of two projects, one in the Bavarian and Bohemian forests (Project-No. 76a-8731.2 - 2000/1) and the second in the Alpine region (Monitoring Network in the Alpine Region for Persistent and other Organic Pollutants: MONARPOP).

1.1 Research Objectives

For the present thesis, monitoring of POP in different matrices was performed in European mountainous areas. The main objective was to understand the differences between active and novel air passive sampling techniques in order to achieve a deeper understanding and to further develop the use of these novel techniques in remote areas. The application of two bioassays was performed with the purpose to determine potential biological effects generated by the accumulation of POP in these regions, effects not accounted for the analytical determinations.

To achieve these objectives, the present work addresses the following issues:

a) Air monitoring of POP in remote mountainous areas.

b) Comparison of alternative methods of air sampling for POP: SPMD and coated stir bars versus classical low volume active sampling.

c) Monitoring of natural compartments indicating short and long term loading: spruce needles, humus and mineral soil.

d) Reinforcement and complementary of the analytical determinations by applying two bioassays to establish the total potential of the samples as AhR inducers and endocrine disrupters.

2. BACKGROUND AND METHODS

2.1 Family of compounds studied

According to the Stockholm Convention, POP are defined based on the following properties: a) persistence, b) bioaccumulation c) long-range environmental transport and d) adverse effects such as toxicity and ecotoxicity to humans or the environment. The identification criteria of new POP are also based on these above mentioned points (Stockholm Convention, 2001).

This work studies the classical POP such as polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/F) and polychlorinated biphenyls (PCB), but also an extended list of pesticides, as well as the more relevant polynuclear aromatic hydrocarbons (PAH).

2.1.1 Polychlorinated Dibenzo-*p*-dioxins, Dibenzofurans and Polychlorinated Biphenyls

These compounds are chlorinated aromatic molecules. The degree and position of the chlorine substituents determine the properties of each compound. For example, a higher degree of chlorination increases the octanol-air partition coefficient (K_{oa}) value, an important property to indicate the compound affinity between air and a lipophilic phase. More chlorine substituents in the molecule cause a decrease in the compound volatility.

PCDD/F are structures with two aromatic rings bound through a third oxygenated ring. Two oxygens are exhibited in PCDD and one in PCDF (Figure 2-1). From the 75 PCDD and 135 PCDF, only 7 and 10 respectively, are of importance regarding their toxic effects. The planar configuration of these compounds favours the binding to the AhR. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic compound of the family and therefore used as reference compound in dose-response curves and for the calculation of toxicity equivalency factors (TEFs) for other compounds. The physicochemical properties of this family, named colloquially dioxins, are taken from Harner *et al*., (2000) and Chen *et al*., (2002).

PCB are biphenyl molecules substituted with chlorine (Figure 2-1). PCB are less toxic than PCDD/F but they are environmentally present in higher amounts. In this work, PCB are numbered according to the IUPAC nomenclature (Ballschmiter and Zell, 1980). From the 209 possible chlorine substitutions in the biphenyl molecule, compounds without chlorine in ortho

positions tend to a planar configuration. For instance, the non-ortho PCB, also called planar PCB are more potentially active than ortho PCB and thus, exhibit more affinity for the AhR. 12 of the 209 congeners are considered toxic, the most toxic being the PCB 126. The compounds analysed in this work are the indicator PCB (28, 52, 101, 138, 153, 180), which are exhibited at higher environmental levels, the non-ortho PCB (77, 81, 126, 169), which are dioxin-like compounds and thus the more reactive, and the mono-ortho PCB (105, 114, 118, 123, 156, 157, 167, 189) that follow in reactivity the non-ortho PCB group. The physicochemical properties of PCB used in this work are taken from the compilation and evaluation of data properties performed by Li *et al*. (2003).

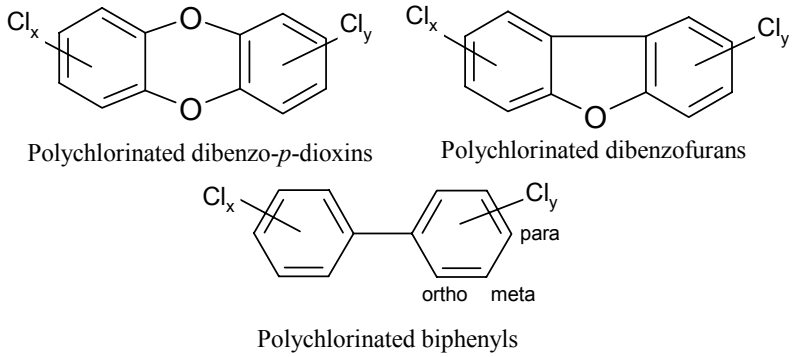


Figure 2-1: Schematic diagram of the general molecular structures of PCDD/F where $1 \leq x + y \leq 8$ and PCB where $1 \leq x + y \leq 10$. The ortho, meta and para positions are indicated in the PCB structure.

The toxicity effect of PCDD/F and PCB can be estimated using Toxic Equivalency Factors (TEF). The TEF values are related to the most toxic compound TCDD which is assigned with a TEF value of 1.0. The TEF value of a compound *i* (TEF_i) multiplied with the concentration of this compound (C_i) in the sample gives the Toxic Equivalent (TEQ) value. Thus, the weighted sum of TEF_i of the n compounds present in the sample gives the TEQ for the whole sample as schematized below.

$$\sum_{i=1}^{i=n} C_i \times TEF_i = TEQ$$

The TEF values used in the current work correspond to the international TEF values evaluated by the World Health Organization (WHO) called also WHO-TEF. These values are taken from the TEF re-evaluation assessed for the WHO in 1997 (Van den Berg *et al.*, 1998).

2.1.2 Organochlorine Pesticides

Hexachlorocyclohexane (HCH) isomers were produced commercially, the technical mixtures containing around 60-70 % α-, 5-12 % β-, 10-12 % γ-, 6-10 % δ- and 3-4 % of ε-isomer (Sittig, 1985). These hexacyclic structures are non planar and their isomer reactivity is determined by the amount of chlorine substituents in axial or equatorial orientation around the ring. δ-HCH has one, α-HCH two and γ-HCH three axial chlorines, respectively. The β-isomer has all its chlorines in the equatorial orientation. Axial chlorines cause more compound reactivity, the γ-isomer being the most reactive of all of them (Keith, 1997) and the one with insecticide properties. Lindane is the commercial name of the insecticide that has 99 % of the γ-isomer.

Hexachlorobenzene (HCB) sources are not limited to its past use as a fungicide, but it is still generated in the organic chemical production as a by-product and it is an impurity in diverse chemicals. It is also produced during waste incineration. In the atmosphere with hydroxyl radicals, it has an estimated half life of 2 years and is usually present in the vapour phase. It is strongly absorbed in soils and sediments (Pesticide Data Sheets). Pentachloroanisole (PCAS) is formed by methylation of pentachlorophenol and can also be produced as a transformation product of HCB via pentachlorobenzene (PeCB) as intermediate. Octachlorostyrene (OCS) is a secondary product in the industrial production of tetrachlorethene and tetrachloromethane, without known utilization.

Technical chlordane (CHL) is composed of 60-85 % of the cis- and trans-stereoisomers cis-CHL and trans-CHL, where cis-CHL is more persistent than trans-CHL. Chlordane has low mobility in the soil matrix, where it is absorbed. Oxychlordane is the main metabolite of chlordane and is even more toxic than the parent material. The commercial mixture contains heptachlor (HC) as a minor constituent (up to 10 %) and cis- and trans-nonachlor. Heptachlor is an insecticide restricted to the use in power transformers to control ants. The hydrolysis of heptachlor in soils is considerable, degrading to 1-hydroxychlordene, cis- and trans-heptachloroepoxide (cis-HCE, trans-HCE) and an unidentified metabolite. Cis-HCE is a persistent metabolite and also bioconcentrates in the food chain (Keith, 1997).

Aldrin has high acute toxicity and tends to degrade to dieldrin, a non- systemic pesticide. The persistence of dieldrin in soils is longer than 7 years. Endrin is a stereoisomer of dieldrin. Endrin is characterised by a high persistence in the soil matrix, up to 14 years. The technical

endosulfan, used as a pesticide until today is composed of at least 94 % of α- and β- isomers with a ratio of 7:3, respectively.

The 6 DDT compounds, p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD p,p'-DDE and o,p'-DDE, are adsorbed strongly in soil. Standard technical mixtures of the DDT pesticide consist of around 70 % p,p'-DDT and 15 % o,p'-DDT. DDE is an impurity of the pesticide DDT. DDD and DDE are also biodegradation products of DDT (Keith, 1997). Chlorine pesticides such as methoxychlor had a boom in usage after the DDT banning in the 70s until the middle of the 90s, when actions to ban it started due to its toxicity. Methoxychlor and mirex exhibit high lipid solubility, a high stability and high toxicity, and are therefore included into the list of pesticides to monitor. Some physicochemical properties of the pesticides analysed can be found in section 2.1.4.

2.1.3 Polynuclear Aromatic Hydrocarbons

Polynuclear aromatic hydrocarbons are composed of aromatic rings fused together by means of sharing a pair of carbon atoms. The rings can be arranged linearly, angularly or clustered. The result is a planar molecular structure. The environmentally significant PAH of the more of 500 known aromatic structures are those molecules which contain two to seven benzene rings. In this group, there is a large number of PAH which differ regarding to the number and position of their aromatic rings. Therefore, they differ in their inherent chemistry. Physical and chemical properties of PAH also vary with molecular weight. For instance, PAH resistance to oxidation, reduction, and vaporization increases with increasing molecular weight, whereas the aqueous solubility of these compounds decreases. As a result, PAH differ in their behaviour, environment spreading and effects on biological systems. PAH can be divided into two groups based on their physicochemical properties and biological characteristics. The low molecular weight PAH (2 to 3 benzene rings) have significant acute toxicity to aquatic organisms, whereas the high molecular weight PAH, with 4 to 7 rings do not. However, several members of the high molecular weight PAH have been known to be carcinogenic. The low molecular weight PAH present a low affinity for particle adsorption, whereas higher molecular weight PAH have a strong adsorption affinity onto particles surfaces.

The PAH analysed in this work are the 16 compounds called priority pollutants by the United States Environmental Protection Agency (EPA PAH): Naphthalene (NA), Acenaphthylene

(ACL), Acenaphthene (AC), Fluorene (FL), Phenanthrene (PHE), Anthracene (AN), Fluoranthene (FA), Pyrene (PY), Benz(a)anthracene (BaA), Chrysene (Chr), Benzo[b]fluoranthene (BbFA), Benzo[k]fluoranthene (BkFA), Benzo[a]pyrene (BaP), Indeno[1,2,3-cd]pyrene (IP), Dibenz[a,h]anthracene (DBahA). Three more are added due to their potential toxicity: Benzo[e]pyrene (BeP), Perylene (PE) and Benzo(b)naphtho(1,2-d)thiophene. Further information is given in the following section 2.1.4.

2.1.4 Physicochemical properties

The uptake of compounds is not only related to the sampler characteristics and the environmental conditions, but to the physicochemical properties of the compounds as well. POP undergo dry and wet deposition. Dry deposition can be either gaseous or particulate, depending mainly on the vapour pressures of the compounds, particulate size and wind speed. Wet deposition is the washout of chemicals associated to the vapour phase and particulate material during precipitation and is related to the water-air partition coefficient and washout ratio (Barber *et al*., 2004). As a consequence, the particle size distribution plays an important role in both depositional processes, where the compounds can either be associated completely or partially to the particulate matter or can stay exclusively in the gaseous phase. A combination of compound properties and environmental aspects determines the gas-particle partitioning for a given compound, a key factor during the sampling uptake. The compound distribution in the gaseous-particulate phases determines the availability of the compound to be transported into the passive sampling device, directly or after desorption processes from the particulate material.

In this section, some selected properties of interest are given for the posterior analysis of compound uptake in the passive samplers. The molecular structure and molecular weight (MW) are provided. These properties are of importance because they influence the compound diffusivity. The diffusivity gives the facility of one substance to be transported within another. For example, compounds with higher MW and rigid structures tend to have a lower diffusivity through membranes (Reynolds *et al*., 1990; Huckins *et al*., 2006). The octanol-air partition coefficient (K_{oa}) indicates the compound affinity between atmospheric and organic phases and it can be resembled to the affinity between air and the triolein phase in SPMD (K_{sa}) or air and the coating polymer of stir bars. K_{oa} is defined as the ratio of the compound concentration in 1-octanol (mol/L) to the compound concentration in air at equilibrium (mol/L). Therefore, the definition used in the current work, implies a dimensionless octanol-air partition coefficient.

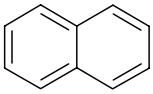
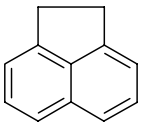
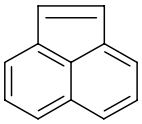
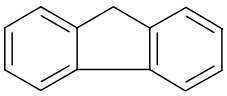
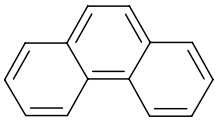
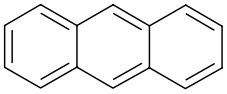
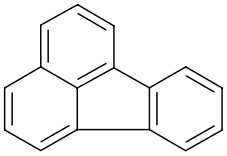
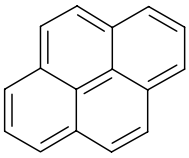
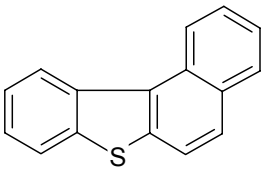
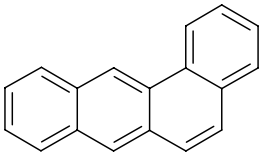
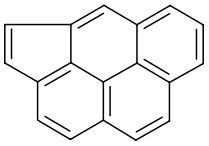
Higher K_{oa} values mean higher affinity for the fatty phase. The K_{oa} is also closely related to the gas-particle partitioning of organic compounds in air (Finizio *et al*., 1997). Organic compounds with high K_{oa} values are more prone to be absorbed into particulate matter. The particle-partitioning of POP is influenced by the source of the pollutants. PCDD/F and PAH, originated in combustion and incineration processes, are from the beginning partially associated with particles, whereas PCB and OCP in the gaseous phase are exchanged with atmospheric aerosol after volatilization. The fraction of compounds available as gaseous is also influenced by other factors, such as the environmental temperature. It is also observed that even the size of particles to which the compounds are attached varies with the temperature, and thus with the season (Kiss *et al*., 1998).

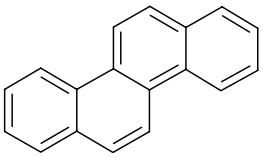
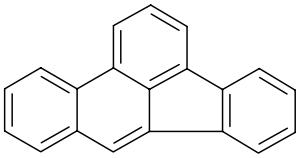
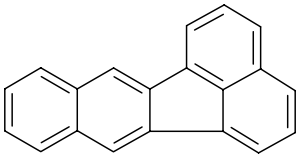
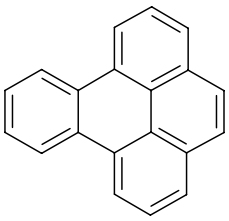
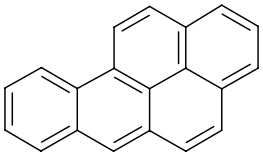
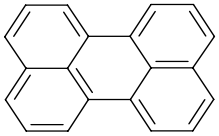
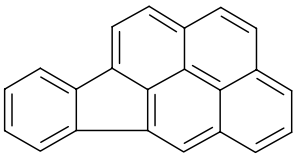
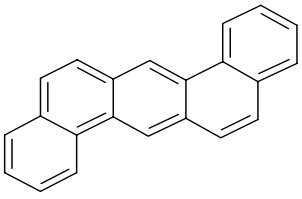
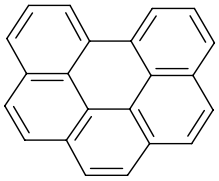
A study performed by Lohmann and co-workers (2000) showed, in general terms, the following percentages of particle bounded compounds: PCB ≤ 50 %, Tetra to PentaCDD/F from 7 to 98 %, HexaCDD/F from 69 to 100 %, PAH with MW ≤ 200 mainly associated to the gas phase (≤ 25 % particle bounded fraction) and PAH with MW ≥ 252 almost completely particle bounded. The gaseous percentage of the compound is the readily available fraction able to diffuse through the membrane of the passive samplers. For the PAH the gaseous-particle percentage distribution is listed for each compound, if known (Table 2-1). The K_{oa} values for PAH are also given in table 2-1. These values were calculated from the dimensionless Henry's law constant (K_H) and the octanol-water partition coefficient (Odabasi *et al*., 2006).

For OCP, the Henry's law constant is given as an indicator of the affinity of the pesticide to the gaseous phase in relation to the aqueous phase (Table 2-2). Higher K_H values imply more atmospheric partitioning, therefore greater availability to undergo atmospheric transport. The tendency of a compound to escape from one phase (fugacity) is calculated based on the Henry's law constant and K_{oa} (Mackay, 1979; Schramm *et al*., 1987). The calculation of the fugacity is shown in Appendix I. The OCP data are obtained from the Pesticide Data Sheets. Otherwise, sources are indicated at the end of the table 2-2.

The abbreviations used for PAH and OCP compounds in the current work are given in the tables 2-1 and 2-2, respectively.

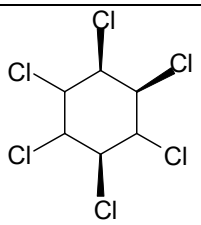
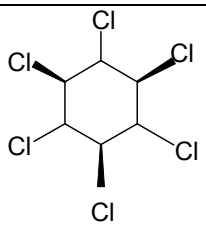
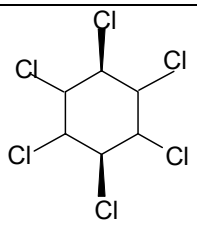
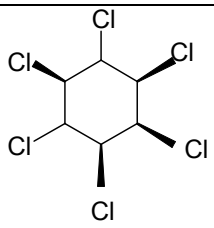
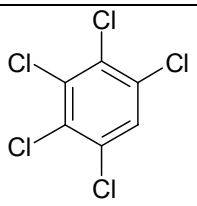
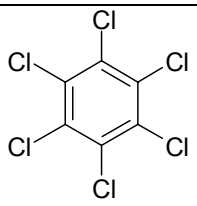
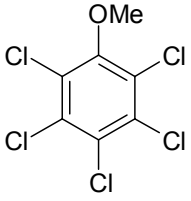
Table 2-1: Properties of some PAH. Molecular structure, MW (g mol^{-1}), log K_{oa} at 25°C and amount of PAH in the particulate phase (%).

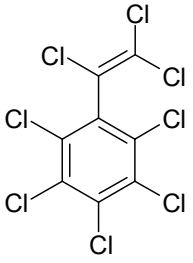
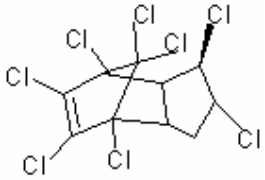
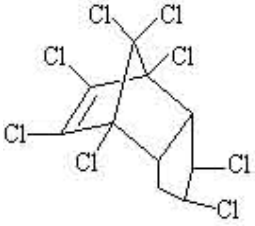
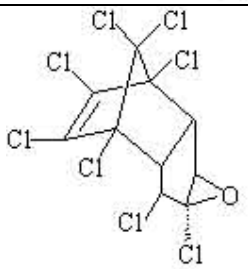
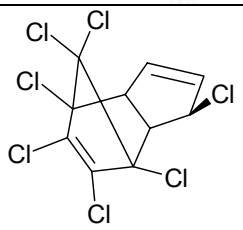
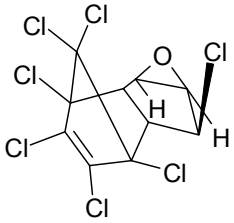
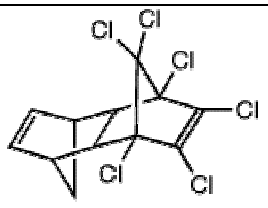
Compound	Molecular structure	MW (g mol^{-1})	log K_{oa}	Particulate phase (%)
Naphtalene (NA)		128.2	-	0.0 %[a]
Acenaphthene (AC)		154.2	6.52	-
Acenaphthylene (ACL)		152.2	6.34	-
Fluorene (FL)		166.2	6.90	-
Phenanthrene (PHE)		178.2	7.68	1.9 %[b]
Anthracene (AN)		178.2	-	0.5 %[a]
Fluoranthene (FA)		202.3	8.76	19.1 %[b]
Pyrene (PY)		202.1	-	29.6 %[b]
Benzo(b)naphtha (1,2-d)thiophene (BbnT)		234.3	-	-
Benz(a)anthracene (BaA)		228.3	10.28	62.7 %[b]
Cyclopenta(cd)pyrene (CPP)		226.3	-	-

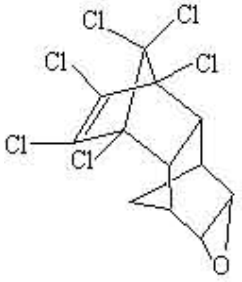
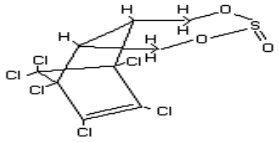
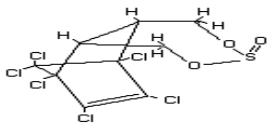
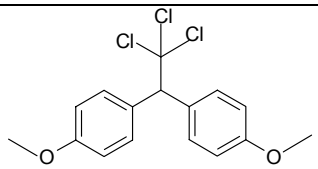
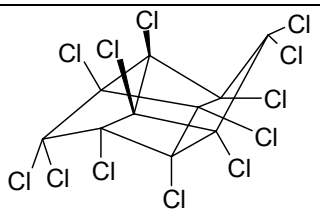
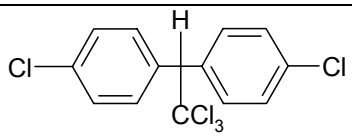
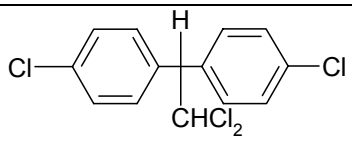
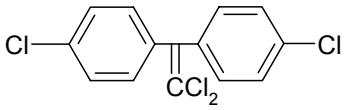
Compound	Molecular structure	MW (g mol^{-1})	log K_{oa}	Particulate phase (%)
Chrysene (Chr)		228.3	10.30	99 %[c]
Benzo(b)fluoranthene (BbFA)		252.3	11.34	92.3 %[b]
Benzo(k)fluoranthene (BkFA)		252.3	11.37	-
Benzo(e)pyrene (BeP)		252.3	-	-
Benzo(a)pyrene (BaP)		252.3	11.56	98.3 %[a]
Perylene (PE)		252.3	-	90.0 %[a]
Indeno(1,2,3-cd)pyrene (IP)		276.3	12.43	-
Dibenz(a,h)anthracene (DBahA)		278.3	12.59	100 %[b]
Benzo(g,h,i)perylene (BghiP)		276.4	12.55	100 %[b]

a) Arey *et al*., 1987 b) Horstmann and McLachlan, 1998 c) Thrane and Mikalsen, 1981.

Table 2-2: Properties of some OCP. Molecular structure, MW (g mol^{-1}), log K_{oa} at 5°C and K_H (Pa m^3 mol^{-1}) at 25°C.

Compound	Molecular structure	MW (g mol^{-1})	log K_{oa}	K_H (Pa m^3 mol^{-1})
α-Hexachlorocyclohexane (α-HCH) $C_6H_6Cl_6$		290.83	8.39[a]	0.652[a]
β-Hexachlorocyclohexane (β-HCH) $C_6H_6Cl_6$		290.83	10.06[a]	0.0374[a]
γ - Hexachlorocyclohexane (γ-HCH) $C_6H_6Cl_6$ (Lindane > 99 % γ-HCH)		290.83	8.67[a]	0.272[a]
δ - Hexachlorocyclohexane (δ-HCH) $C_6H_6Cl_6$		290.83	-	-
Pentachlorobenzene (PeCB) C_6HCl_5		250.32	7.18[b]	72[b]
Hexachlorobenzene (HCB) C_6Cl_6		284.79	8.09[b]	65[b]
Pentachloroanisole (PCAS) $C_7Cl_5OH_3$		280.38	-	-

Compound	Molecular structure	MW (g mol^{-1})	log K_{oa}	K_H (Pa m^3 mol^{-1})
Octachlorostyrene (OCS) C_8Cl_8		379.71	-	-
trans-chlordane (trans-CHL) $C_{10}H_6Cl_8$		409.8	8.83[b]	6.8[b]
cis-chlordane (cis-CHL) $C_{10}H_6Cl_8$		409.8	8.83[b]	5.7[b]
oxy-chlordane (oxy-CHL) $C_{10}H_4Cl_8O$		420	-	-
Heptachlor (HC) $C_{10}H_5Cl_7$		373.4	7.76[b]	38[b]
cis-heptachlorepoxide (cis-HCE) $C_{10}H_5Cl_7O$		389.2	8.59[b]	1.7[b]
Aldrin $C_{12}H_8Cl_6$		364.9	8.26[b]	23[b]

Compound	Molecular structure	MW (g mol^{-1})	log K_{oa}	K_H (Pa m^3 mol^{-1})
Dieldrin $C_{12}H_8Cl_6O$		380.9	8.84[b]	1.1[b]
α-Endosulfan $C_9H_6Cl_6O_3S$		406.9	9.81[b]	0.70[b]
β-Endosulfan $C_9H_6Cl_6O_3S$		406.9	9.68[b]	0.045[b]
Methoxychlor $C_{16}H_{15}Cl_3O_2$		345.6	-	1.62
Mirex $C_{10}Cl_{12}$		545.5	-	-
p, p'-DDT $C_{14}H_9Cl_5$		354.5	9.73[b]	1.1[b]
p, p'-DDD $C_{14}H_{10}Cl_4$		320.1	10.03[b]	0.5[b]
p, p'-DDE $C_{14}H_8Cl_4$		318.0	9.70[b]	4.2[b]

a) Xiao *et al*., 2004 b) Shen and Wania, 2005.

2.2 Sampling Methods

The sampling methods are described generally, but practical considerations, related specifically to this work, are also included.

2.2.1 Active air sampling

This kind of sampling is defined as "methods that require physical intervention or external energy input" (Huckins *et al*., 2006). Low volume active sampling (LowVol) is the kind of active method chosen here, because it allows the collection of compounds for periods of some months, in contrast to high or medium volume sampling designed to work for some hours or days. In this way, it is possible to determine the time weighted average (TWA) concentrations e.g. over different seasons or monthly. In an air volume active sampler, air is pumped through a collector cartridge filled with XAD amberlite topped with a fibreglass filter (Figure 2-2), where organic compounds are accumulated. This sampling method comprises the particulate (fibreglass filter) as well as the gas phase (XAD resin) of the compound. The volume of pumped air and the direction of the air uptake are modifiable and known. To assure accuracy and reliability of the data, a high degree of maintenance is necessary. It requires calibration of the sampler following adequate quality assurance and control procedures, leading to high operation costs. Figure 2-2 depicts a LowVol active sampler (Digitel blower, DPA96) deployed at the Bavarian Forest. Note the 5 different cartridges which allow the sampler to collect compounds discriminated by the predominant winds: North-West (NW), South-West (SW), North-East (NE), South-East (SE) directions and calm. The pump is switched on/off through the different cartridges according to the direction of the dominant wind.

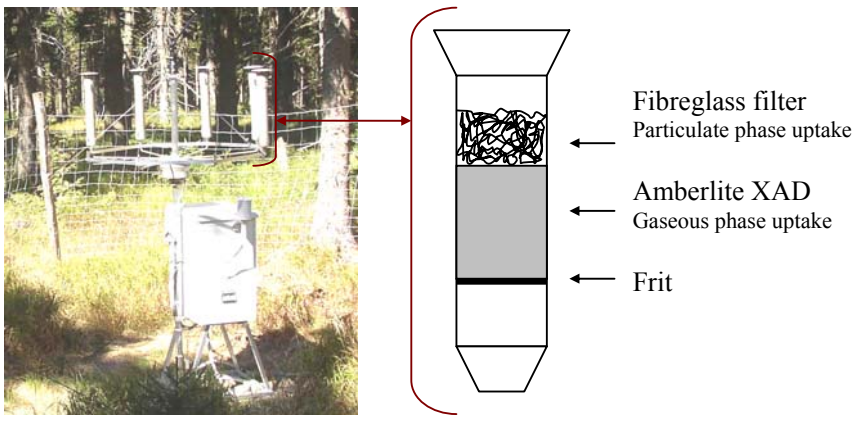


Figure 2-2: Low volume active sampler and schematic representation of a cartridge design.

2.2.2 Passive air sampling

Huckins and co-workers defined passive samplers as "human-made devices where sample collection occurs in a completely passive manner" (Huckins *et al*., 2006). The process of accumulation of compounds into these devices is based on diffusion or sorption of chemicals from one matrix to another of higher compound affinity. The advantage of the passive air samplers (PAS) in comparison to the active samplers is their simple operation, requiring no power supply and allowing long deployment times. One important advantage of the integrative uptake achieved at long deployment periods is the detection of compounds otherwise unnoticed by active air sampling over a short period. Within this work, two types of PAS are defined:

a) Natural passive samplers such as spruce needles (*Picea abies (L.) Karst*)
b) Human-made samplers, such as SPMD and coated stir bars.

The man-made samplers have the advantage of their standardized design, in contrast to the natural passive samplers, whose uptake capacity depends on the growing conditions, geographical site and age of the plants. Human-made passive devices can also be deployed in remote areas where there is no vegetation. On the other hand, the cost of the natural passive samplers lies only in the plant collection.

2.2.2.1 Needles as a natural passive sampler

The uptake of POP is strongly dependent on the vegetation species chosen for the biomonitoring (Böhme *et al*., 1999). Evergreen conifer forests are an excellent sink of organic compounds due to the lipophilic properties of the needles cuticle and their great superficial area. Spruce trees have good collection efficiency for particles and a good interception of precipitation, and these factors make this species suitable as a passive sampler (Umlauf and McLachlan, 1994). The needles accumulate compounds not only from the gaseous, but also from the particulate phase.

This passive sampler has been roughly considered as a single compartment where a mass balance can be applied considering only uptake and elimination processes. However, Umlauf *et al*. (1994) have obtained results that express concern about considering needles as a single well-mixed compartment. Tolls and McLachlan, (1994) suggested a two-compartment system as a model in the uptake of lipophilic compounds thorough the leaf.

The mass transfer of the compounds from the air into the leaf is still not well-known in detail but the transport processes are the following ones: a) diffusion transport (mainly Eddy diffusion) through the bulk air to the air laminar layer that surrounds the canopy b) transport

through the laminar layer to the surface of the leaf and c) diffusive transport from the surface of the leaf into it. The uptake into the leaf is a parallel mass transfer through cuticle and stomata, where the mass transfer resistance of the cuticle pathway presents more variability than the stomatal pathway. The differences in cuticle type can imply different permeabilities, thus different mass transfer coefficients for POP (Barber *et al*., 2004).

The accumulation of lipophilic compounds in needles increases with the needle age until senescence starts, and this accumulation is strongly related to the species used as a passive sampler (Hellström *et al*., 2004). The applied method of extraction removes POP accumulated in the cuticle, but not compounds accumulated in the inner compartment of the leaf. It is also important to mention that the vegetation scavenging effect (the uptake and accumulation of compounds into the vegetation) is dependant of the current season. There seems to be greater scavenging effects in the spring/summer months in comparison to the autumn/winter ones (Lee *et al*., 1999). Due to this, it is a key issue to sample the different year classes simultaneously so as to get independency from possible seasonal variations present in the needles (Kylin *et al*., 2003). The vegetation growth is also an important factor related to the scavenging effect of the vegetation. Foliar biomass of the sampling sites is determined in order to detect possible influences of this factor (Jaward *et al*., 2005). To estimate the foliar biomass, the stand biomass is measured.

2.2.2.2 SPMD as passive air sampler

SPMD are integrative passive samplers, as they accumulate compounds during the sampling time until reaching an equilibrium stage. The uptake is based on the contaminant concentration difference between the air and the semi-permeable device, but only small compounds are able to penetrate through the membrane. As a consequence, contaminants associated with particulate material are mainly not absorbed, such that toxic compounds within this group can be underestimated when using SPMD.

The sampler used in this work consists of a tube composed of a polymeric membrane (low density polyethylene: LDPE) that encloses a lipophilic solvent: triolein (1,2,3-tris[cis-9-octadecenoylglycerol]glycerol). The LDPE membrane has transient polymeric cavities of about 10 A° diameter. This size exclusion avoids the diffusion of triolein out of the membrane and simultaneously limits the uptake of large organic molecules through it (Figure 2-3). Triolein is a natural triglyceride (MW 885.4) that solidifies at -4°C, is able to dissolve solid fats and does not react with absorbed pollutants. These characteristics make SPMD suitable for the uptake of hydrophobic organic chemicals with molecular weights lower than 600

Daltons. As triolein is found as a natural storage lipid in most organisms and the size exclusion of molecules able to be transported through biological membranes is around 9.8 A° (Opperhuizen *et al*., 1985), SPMD are expected to mimic roughly the accumulation in living organisms.

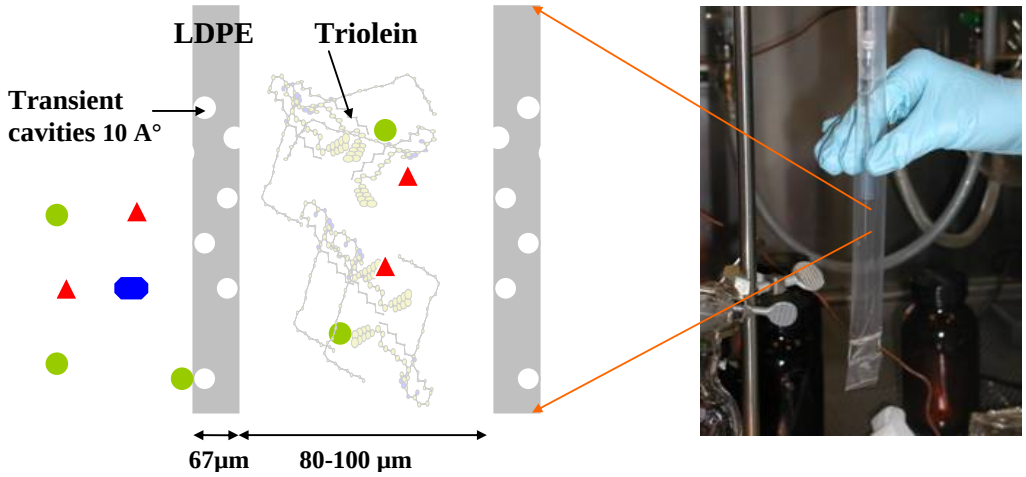


Figure 2-3: Schematic representation and SPMD preparation at the laboratory.

According to Bartkow *et al.* (2005), the exchange of compounds between the passive device and the air can be divided in three steps

1) a linear uptake stage where the uptake is proportional to the concentration of the compound in the device surroundings
2) a curvilinear stage where the elimination of the absorbed compound from the device achieves importance and
3) a steady state stage where the uptake/elimination of the analyte in the device approaches equilibrium (Figure 2-4).

When the device is operating in the linear uptake stage, the sampler is called kinetic sampler. For devices designed to operate in the kinetic stage, the exposure time is a key factor in the passive sampler performance. Other parameters of importance to define the accumulation capacity of passive samplers are film thickness, sampler area (A), sampler volume (V) and the sampler area to volume ratio A/V.

In this work, SPMD are designed as LDPE membranes (length 23 cm, wide 2.5 cm, thickness ≈ 67.5 µm) filled with 0.7 ml triolein 99 % (Sigma-Aldrich, Taufkirchen, Germany) that were heat sealed under inert gas conditions (N_2) in a glove chamber to avoid contaminations. The sampler area (A) is ≈ 115 cm^2 and the area to volume ratio A/V≈ 165 cm^{-1}.

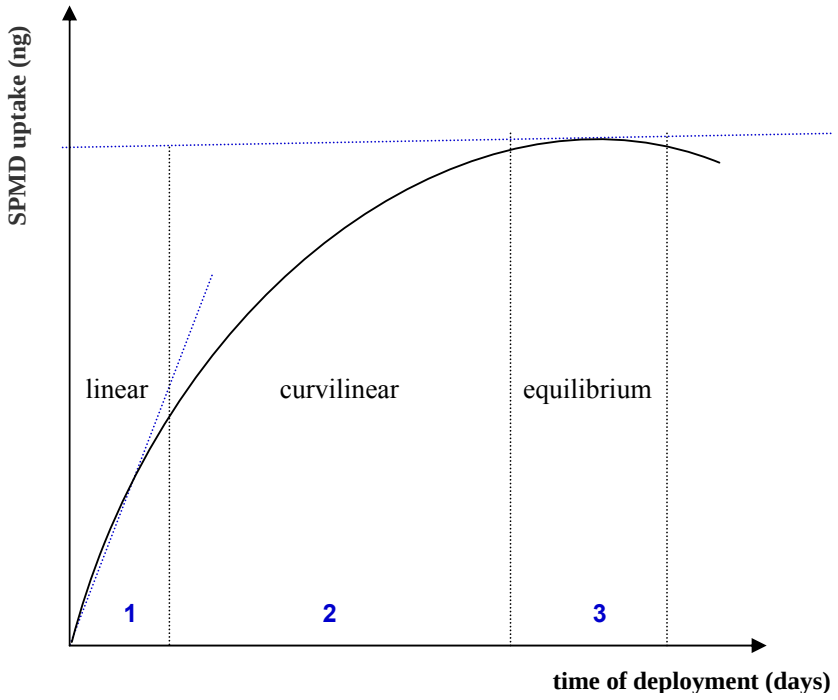


Figure 2-4: Uptake stages in SPMD as a function of the time.

The uptake of compounds in these devices faces three barriers. First, the boundary air layer is the laminar layer that the compounds have to diffuse through to reach the surface of the sampler. Second, the transient cavity structure of the membrane acts as a diffusive and permeative barrier. At this point, compounds attached to particles are totally or partially limited in their passage through the membrane, even if they could readily diffuse through it in the gaseous phase. In this case, desorption of compounds from the particles has to be considered as a limiting step. Chemicals transferred through the membrane reaching the internal side have to diffuse through the triolein. This third and last barrier generated by the triolein phase is smaller than the other two resistance barriers and therefore can be neglected. In sum, the compound uptake is restricted mainly by two barriers (boundary air layer and membrane) for gaseous phase compounds.

When the boundary air layer is the main mass transfer barrier, the uptake is under air boundary layer control. Otherwise, when the membrane is the major limiting to the mass transfer, the passive sampler is operating under membrane controlled uptake. In general it can be expected that higher K_{oa} values and more turbulence lead to membrane control uptake. On the other hand, compounds with lower affinity for the triolein phase (lower K_{oa}) and thicker

air-membrane laminar layer are under air boundary layer control. The possibility of a biofilm layer acting as an extra barrier in the compound-SPMD mass transfer is usually not considered. Such an approximation is only valid for the deployment of devices in air but not in aquatic media, where the development of an external biofouling can be significant. In Appendix II, scanning electron microscopies of SPMD surfaces are shown after 1 year aerial deployment in forested areas.

The SPMD data interpretation was generally assumed to be limited to qualitative or semi-quantitative results (Ockenden *et al*., 1998). In the aim to quantify the amount of compounds in air regarding the passive uptake, two simplified models relating air concentrations (C_{air}) and SPMD compound accumulation (C_s) are described below. These models are based on the theory described by Huckins *et al*. (1993, 2006).

Chemical Reaction Kinetics Model (CRK)

If the resistance to the mass transfer is controlled by the boundary layer, we can model the system considering the SPMD device as a one compartment as follows (Figure 2-5):

Figure 2-5: Schematic representation of one compartment model for SPMD. C_{air}: compound concentration in air, k_u: uptake rate constant, C_s: compound concentration within the SPMD, k_e: elimination rate constant.

Applying a mass balance in the SPMD compartment leads to:

$$\frac{\partial C_s}{\partial t} = k_u C_{air} - k_e C_s \qquad \text{Equation 1}$$

Out of this mass balance, the relation between sampling time t, C_s and C_{air} can be calculated. Regrouping and integrating equation 1 in a span of time t gives:

$$\frac{\partial C_s}{(k_u C_{air} - k_e C_s)} = \partial t \Rightarrow \frac{-1}{k_e} \int_0^{c(t)} \frac{\partial C_s}{(\frac{-k_u C_{air}}{k_e} + C_s)} = \int_o^t \partial t \Rightarrow Ln \left(\frac{\frac{-k_u C_{air}}{k_e} + C_s(t)}{\frac{-k_u C_{air}}{k_e}} \right) = -k_e t$$

Applying e^x and regrouping results in:

$$C_s(t) = \frac{k_u C_{air}}{k_e}\left(1 - e^{-k_e t}\right) \Rightarrow C_s(t) = C_{air} \mathrm{K}_{sa}\left(1 - e^{-k_e t}\right) \qquad \text{Equation 2}$$

where K_{sa} is the SPMD-air partition coefficient ($K_{sa} = k_u/k_e$).

When the SPMD is still in the linear stage, the amount of compound released from the SPMD to the air compartment is negligible, thus from equation 1 $k_e C_s \rightarrow 0$

$$\Rightarrow \partial C_s / \partial t \cong k_u C_{air} \qquad \text{Equation 3}$$

Integrating this approximation in a span of time t leads to:

$$C_s(t) = k_u C_{air} t \qquad \text{Linear uptake stage} \qquad \text{Equation 4}$$

When the SPMD has reached equilibrium conditions, such that the concentrations do not change anymore, equation 1 leads to:

$$\partial C_s / \partial t = 0 \Rightarrow k_u C_{air} = k_e C_s \qquad \text{Equation 5}$$

so the compound concentrations in the air and the SPMD are related through the uptake and elimination rate constants:

$$C_{air} = \frac{k_e C_s}{k_u} = \frac{C_s}{\mathrm{K}_{sa}} \qquad \text{Equilibrium conditions} \qquad \text{Equation 6}$$

Mass Transfer Coefficient Model

This model describes the mass transfer in the system by means of mass transfer coefficients. The compound concentrations in the different compartments are schematized in Figure 2-6:

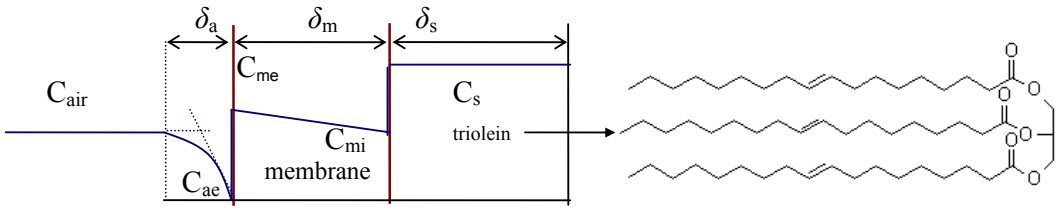


Figure 2-6: Schematic overview of the concentration distribution air-SPMD. C_{air}: compound concentration in air, C_{ae}: compound concentration at the membrane outside boundary layer, C_{me}: compound concentration at the membrane inside boundary layer, C_{mi}: compound concentration at the membrane internal side, C_s: compound concentration in SPMD, δ: effective thickness of each region and their associated subscripts.

Appling Fick´s First law and assuming concentration homogeneity within the device, the flux F (moles s^{-1}) generated is:

$$F = -DA\frac{\partial C}{\partial \delta}, \qquad \text{Equation 1}$$

where D is the diffusivity of the compound ($cm^2\ s^{-1}$) and A is the sectional area (cm^2) where the compound is transferred (membrane surface area), δ (cm) and C (moles cm^{-3}). Substituting the mass transfer coefficients $k_m=D_m/\delta_m$ and $k_a = D_a/\delta_a$ in the equation 1:

$$F = k_m A_{SPMD}(C_{me} - C_{mi}) = k_a A_{SPMD}(C_{air} - C_{ae}) = V_{SPMD}\frac{\partial C_s}{\partial t} \qquad \text{Equation 2}$$

where V_{SPMD} is the sampler volume. Using the equilibrium partition coefficients defined as $K_{ms}=C_{mi}/C_s$, $K_{ma}=C_{me}/C_{ae}$ and $K_{sa}=C_s/C_{air}$ the equation is re-written as a function of the variables of interest C_{air} and C_s, obtaining:

$$F = k_o A_{SPMD}(C_{air}K_{ma} - C_s K_{ms}) = V_{SPMD}\frac{\partial C_s}{\partial t} \qquad \text{Equation 3}$$

where k_o is the total mass transfer coefficient: $\frac{1}{k_o} = \frac{1}{k_m} + \frac{K_{ma}}{k_a}$ Equation 4

Assuming independence and additively of the coefficients and regrouping and integrating the equation 3 we obtain:

$$C_s = C_{air}\frac{K_{ma}}{K_{ms}}(1 - e^{-(k_o A_{SPMD}K_{ms}t/V_{SPMD})}) = C_{air}K_{sa}(1 - e^{-k_T t}) \qquad \text{Equation 5}$$

In this equation the overall uptake rate constant is defined as $k_T = k_o A_{SPMD} K_{ms}/V_{SPMD}$.

Equation 5 describes the uptake stages depicted in Figure 2-3. In short exposure periods, for $t \rightarrow 0$ the limit of the exponential term tends to

$$\lim\left(1 - e^{-(k_o A_{SPMD}K_{ms}t/V_{SPMD})}\right) \rightarrow k_o A_{SPMD}K_{ms}t/V_{SPMD} \qquad \text{Equation 6}$$

Substituting this term in equation 5 and K_{sa} by K_{ma}/K_{ms}, a linear time-dependent function is obtained:

$$C_s = C_{air} \frac{K_{ma}}{K_{ms}} k_o A_{SPMD} K_{ms} t / V_{SPMD} = C_{air} K_{ma} k_o A_{SPMD} t / V_{SPMD}$$ Equation 7

In this linear stage, the uptake of compounds depends on the design of the device (volume and area) and the physicochemical properties of the chemical. The sampling rate R_s is defined as the volume of compound incorporated by the passive sampler per unit of time:

$$R_s = K_{ma} k_o A_{SPMD}$$ Equation 8

and substituting equation 8 in equation 7:

$$C_s = C_{air} R_s t / V_s \Rightarrow R_s = C_s V_s / C_{air} t$$ Equation 9

In this way, in the linear uptake stage, the sampling rate of a compound can be calculated if the SPMD and air concentrations of the compound for a determined exposure time are known. It is interesting to remark that the sampling rate is only a function of the sampler design and physicochemical properties of the compound (equation 8), and thus independent of the compound air concentrations. This implies that for a determined temperature, R_s is a single known value.

For the empirical calculation of sampling rates, $C_s V_s$ is equivalent to the amount of mass N_s sequestered in the SPMD. Substituting N_s in the equation 9 leads to:

$$R_s = N_s / C_{air} t$$ Equation 10

Once sampling rates for compounds have been established for a determined sampling design using equation 10, these sampling rates can be used to estimate the air concentrations of a compound, from the compound uptake into the SPMD and the time of deployment.

In devices deployed enough time to reach steady state conditions the exponential term of equation 5 tends to zero and thus:

$$C_s = C_{air} K_{sa}$$ equilibrium conditions Equation 11

In the equilibrium stage the concentrations in air and SPMD are related exclusively through the SPMD-air partition coefficient K_{sa}, which can be substituted as a first approximation by the octanol-air partition coefficient Koa, obtaining:

$$C_s = C_{air} K_{oa} \Rightarrow C_{air} = C_s / K_{oa} \qquad \text{Equation 12}$$

The equation 12 allows the calculation of the compound air concentration just knowing the K_{oa} value and the amount of chemical accumulated in the device under steady state conditions.

Note that the two models presented above can be related through the constants; elimination rate constant k_e in the kinetic model (equation 2) and overall uptake rate constant k_T in the mass transfer coefficient model (equation 5).

Performance Reference Compounds

Performance reference compounds (PRC) are non-interfering organic chemicals with medium to high affinity for triolein which are added to it before the membrane enclosure and distributed homogeneously in the lipid phase (Huckins *et al*., 2002a). In preference, PRC have to be compounds not exhibited in the environment and due to this, deuterated PAH or ^{13}C-PCB compounds are adequate. The purpose of these compounds is to assess the effect of environmental variables at the different sampling sites. The theory is based on the assumption that the elimination rate (k_e) of PRC is related to the uptake rate of the corresponding native compounds (Petty *et al*., 2000). The release of PRC follows an exponential decay with the exposure time:

$$\frac{\partial C_{PRC}}{\partial t} = -k_e C_{PRC} \Rightarrow \int_{C_{PRC_o}}^{C_{PRC}} \frac{\partial C_{PRC}}{C_{PRC}} = -k_e \int_0^t \partial t \Rightarrow C_{PRC} = C_{PRC_o} e^{-k_e t} \qquad \text{Equation 1}$$

The initial amount of PRC (C_{PRCo}) and the remaining amount after the exposure time (C_{PRC}) are known, such that the elimination rate can be calculated by regrouping the equation 1:

$$k_e = \frac{Ln\left(C_{PRC\,o} / C_{PRC} \right)}{t} \qquad \text{Equation 2}$$

The elimination rate constant k_e is assumed to be identical to the overall uptake rate constant k_T in the mass transfer coefficient model for the native and labelled compound leading to:

$$k_e = {K_{ma} k_o A_{SPMD}} / {V_s K_{sa}} \Rightarrow k_e = {R_s} / {V_s K_{sa}} \Rightarrow k_e V_s K_{sa} = R_s \qquad \text{Equation 3}$$

where R_s was substituted using the equation 8 from the mass transfer model. Equation 3 relates the sampling rate of the compound to the elimination rate obtained "*in situ*" for a determined compound with the PRC. In this way, the effects generated by differences in the exposure sampling conditions can be contemplated. Studies performed by Söderström and Bergqvist, (2004) with PRC demonstrated the influence of the wind speed in the sampling rate. As a consequence, the devices are sheltered in order to avoid differences due to wind effects affecting the turbulence and as a consequence the air-membrane boundary layer thickness (Ockenden *et al*., 2001). Additionally, the sheltering is also important to avoid photodegradation of compounds sensible to UV light. Some other environmental conditions, such as temperature, are also affecting the sampling rate. The use of PRC allows the R_s calculation regarding environmental conditions and even a posterior quantification of TWA compounds in the environment.

The PRC used in the current work are given in table 2-3. These compounds were purchased from LGC Promochem (Wesel, Germany). The decision criteria to choose these compounds were based on achieving measurable PRC losses, in a range between 20-80 % of the initial spiked amount, during the sampling period (Huckins *et al*., 2002b). For this reason, ^{13}C labelled compounds embracing a wide range of vapour pressure and K_{oa} were used. SPMD spiked with PRC were deployed. Simultaneously some of the SPMD were left in hermetic sealed flasks under identical experimental conditions. The unexposed SPMD were used as a laboratory quality control. Further information is found in Zhu *et al*. (2007).

Table 2-3: ^{13}C labelled PCDD and PAH used as PRC.

PRC PCDD-$^{13}C_{12}$	*PRC PAH-$^{13}C_x$*
2,3-DiCDD-$^{13}C_{12}$	Anthracene-$^{13}C_6$
2,3,7-TrCDD-$^{13}C_{12}$	Benzo(a)pyrene-$^{13}C_4$
1,2,3,7,8-PeCDD-$^{13}C_{12}$	Benzo(g,h,i)perylene-$^{13}C_{12}$
1,2,3,7,8,9-HxCDD-$^{13}C_{12}$	-
1,2,3,4,7,8,9-HpCDD-$^{13}C_{12}$	-

2.2.2.3 Coated stir bar as passive air sampler

Coated stir bars (CSB) were first introduced by Arthur and Pawliszyn in 1990. The device design is a rod covered with a coating of a polymeric material (Figure 2-7), able to retain the compounds by dissolution or partitioning (sorption). In this way, these PAS comprise only a single phase of a coating material. The polymer chosen is polydimethylsiloxane (PDMS) with the characteristics of being a rubber-like, homogeneous and non-porous material, in which the chemicals can be dissolved (Baltussen *et al.*, 2002). This material satisfies the requisite of "zero sink". This means that the analyte concentration at the air-polymer interface remains close to zero during the sampling period, avoiding interferences in the sampling rate of additional analyte (Chen and Pawliszyn, 2003).

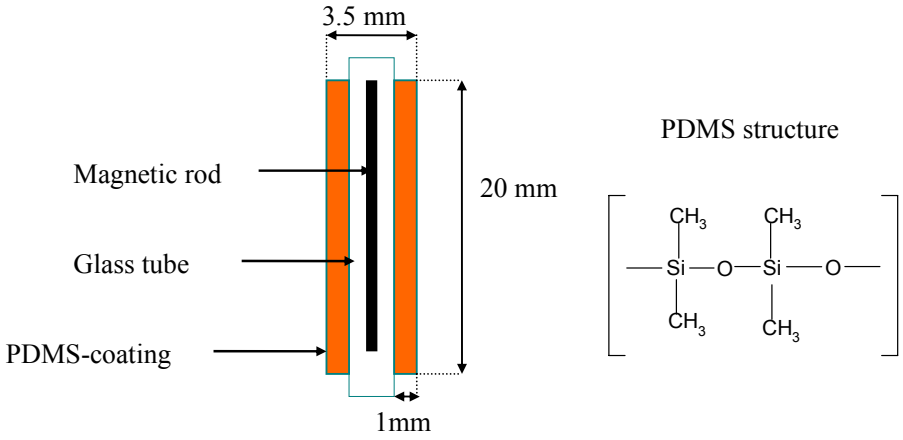


Figure 2-7: Coated stir bar scheme and PDMS polymeric structure.

The advantage of using CSB is that, unlike other PAS, they do not require a clean-up step. The dissolved compounds can be analysed by solid-phase micro extraction (SPME) for which they are desorbed thermically under an inert gas stream coupled to a gas chromatograph followed by a mass spectrometer.

The coated stir bars used for this study have a length of 20 mm and a PDMS film thickness of 1.0 mm. They were acquired with the commercial name of Twisters^{TM} from Gerstel (Mülheim, Germany). The mean diameter is approximately 3.5 mm and this result in an effective area of A ≈ 220 mm^2 and an A to V ratio of A/V≈ 1.4 mm^{-1}. This means that the effective area of the SPMD used for this study is more than 50 times larger than the PDMS coating area of the CSB. On the other hand, diffusion coefficients of organic substances in the PDMS coating are higher than in the LDPE membrane (Rusina *et al.*, 2007). As a consequence, coated stir bars exhibit lower resistance to the molecular transport of organic compounds through the membrane than SPMD. Due to this, the sampling rate is more likely

to be controlled by the membrane in SPMD with compounds of intermediate K_{oa} values, whereas in coated stir bars, these compounds exhibit a lower resistance through the coating that favours a control by the air boundary layer. Table 2-4 summarizes selected properties of LDPE and PDMS, the interface polymers in SPMD and coated stir bars, respectively.

Table 2-4: PDMS and LDPE selected properties. T_g: glass-transition temperature. Rusina *et al*., 2007.

Polymer	*Thickness (mm)*	*Density (g cm^{-3})*	*Melting point (°C)*	*T_g (°C)*	*Structural unit*
PDMS	0.4	1.15	150-200	-120	$[-Si(CH_3)2-]_nO-$
LDPE	0.066	0.915	120	-40	$[-CH_2-CH_2-]_n$

Coated stir bars present as an advantage lower costs at the laboratory in comparison with SPMD regarding usage of time and solvents during the sample preparation as well as the cost of the device itself. Coated bars can be re-used after conditioning. As a disadvantage, the lack of standard procedures and experience in air monitoring with these one-phase devices has to be considered.

Mass Transfer Model

The passive sampling process is described by Fick´s first law and schematized in Figure 2-8.

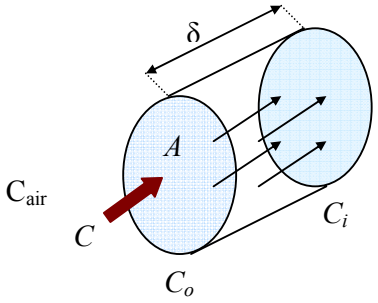


Figure 2-8: Scheme of the mass transfer in passive samplers (modified from Gorecki and Namienski, 2002).

Applying the Fick´s first law and integrating over a time t, leads to:

$$\frac{\partial M_s}{\partial t} = \frac{DA(C_i - C_o)}{\delta} \Rightarrow M_s = \frac{DAC_{air}}{\delta} t \,, \qquad \text{Equation 1}$$

where M_s is the analyte mass, C_i is the analyte concentration inside the sorption coating and C_o is the concentration at the membrane interface. The other terms were defined earlier for

SPMD. The simplification that the compound concentration C_i at the diffusive layer is equal to the air concentration of the compound (C_{air}) was applied and also the "zero sink" principle, i.e. $C_0 \rightarrow 0$. This equation allows the calculation of the air concentrations knowing the amount of analyte absorbed in the coating, compound diffusivity and the device design parameters A and δ. This time-related linear uptake allows the calculation of TWA concentration of compounds in these devices.

2.2.3 Soil as a POP accumulation matrix

Soil can be schematically described as a three compartment model as follows (Harner *et. al*, 2001):

a) a top layer that acts as a soil-air exchange layer,
b) a buffer layer where chemicals are leached down from the top and diffused up from the bottom and
c) a reservoir layer where chemicals can be accumulated for long terms.

For this study, soil samples were taken at two depths, comprising the humus layer and the deeper mineral layer. The humus samples were collected by taking the whole humus found within a 30 x 30 cm metal frame. 10 pits along a 5 x 30 m rectangular grid were randomly collected per sampling site and mixed as a pooled sample. After taking the humus layer, the uppermost 10 cm soil beneath was defined as the mineral soil. Using the compartment model described above by Harner *et. al* (2001), the humus samples belong to the first two mentioned layers, but the mineral soil not always corresponds completely to the reservoir layer. The fate of POP is highly influenced by the organic content in soil and the soil texture. Due to this the comparison of data among different sampling sites has to be done considering these properties (Daly *et al*., 2007). The organic carbon content was determined by elementary analysis measurement carried out at the Institute of Soil Ecology in the Soil Functions and Material fluxes group (Dr. A. Zsolnay) in the German Research Center for Environmental Health.

2.3 Sampling Sites

2.3.1 Bavarian and Bohemian forest sites

Sampling took place in a mountainous area at the Bavarian and Bohemian forests (Central Europe). The four sampling sites Mitterfels (M), Ruckowitzschachten (R), Haidel (H) and Boubin (B) (Figure 2-9) are of similar characteristics regarding altitude, temperature regime, forest stand characteristic and as far as possible, similar contaminant sources (Kirchner *et al*., 2006).

The forest stands (foliar biomass) are at Mitterfels 40 m^2/ha, at Haidel 46 m^2/ha, at Boubin 54 m^2/ha and at Ruckowitzschachten 60 m^2/ha (measurement error ± 3 m^2/ha). The forest composition at Boubin and Mitterfels is based mainly of spruce stands (*Picea abies (L.) Karst*), but deciduous species such as beech, rowan berry and ash maple are also found (< 10 %) at Haidel. In contrast, Ruckowitzschachten has approximately the same amount of beech and spruce stands. The solar irradiation is determined by the geographical characteristics and decreases in the following order: Mitterfels (1030 m a.s.l.) and Haidel (1160 m a.s.l.), both hills with round ends, Ruckowitzschachten (slope facing to the west, 1130 m a.s.l.) and Boubin (slope facing to the north, 1300 m a.s.l.). The sampling sites are characterized by soils of granite and gneiss bed rock and belong to the same altitudinal zone. The soil texture classes corresponding to the sampling in mineral soils are classified as sandy loam or silt loam textures, except for Mitterfels, that has silt-loamy sand and loamy sand textures.

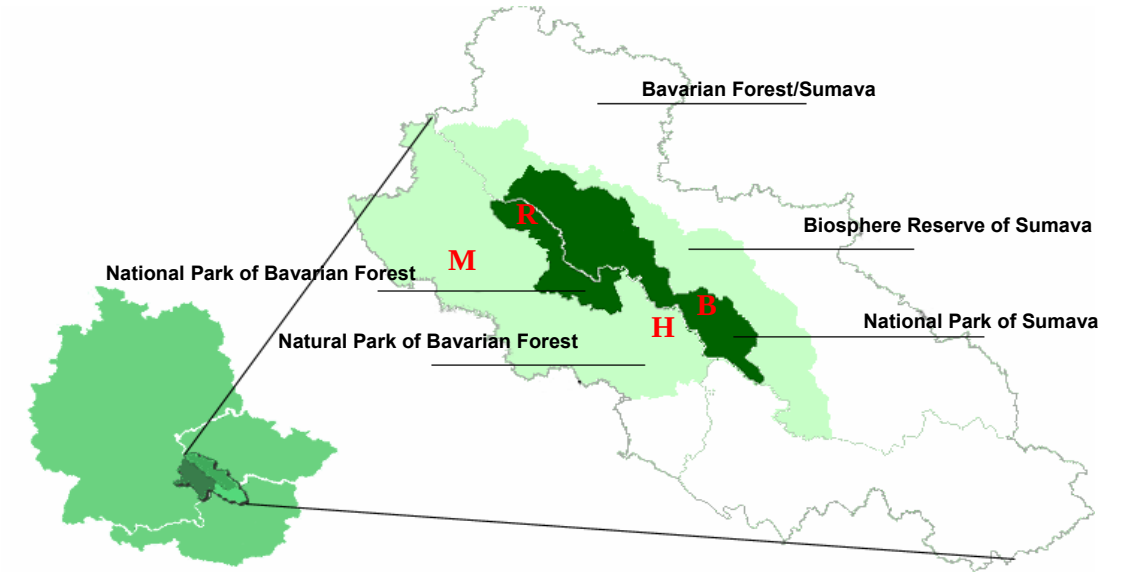


Figure 2-9: Sampling sites at the Bavarian and Bohemian forests. H: Haidel, B: Boubin, R: Ruckowitzschachten and M: Mitterfels.

The passive samplers were deployed into Stevenson screen boxes of untreated wood (50 cm x 50 cm x 40 cm) placed at 3 m above ground level in forest clearings (Figure 2-10). Air-flow turbulences influence the compound mass transfer in SPMD, thus the Stevenson screens were used to minimize this effect. Further, these deployment devices hinder wet deposition and direct irradiation of sun light, but allow a good air flow through them. Inside each Stevenson screen box, four metal frames were positioned hanging in parallel. Each metal frame can contain up to five SPMD, and coated stir bars were magnetically attached to the frames (Figure 2-10).

Coated stir bars and SPMD were deployed simultaneously for ½ and 1 ½ years and collected in October 2003 and October 2004, respectively. The spruce needles were collected with ½, 1½ and 2½ years of age in October 2003 and October 2004.

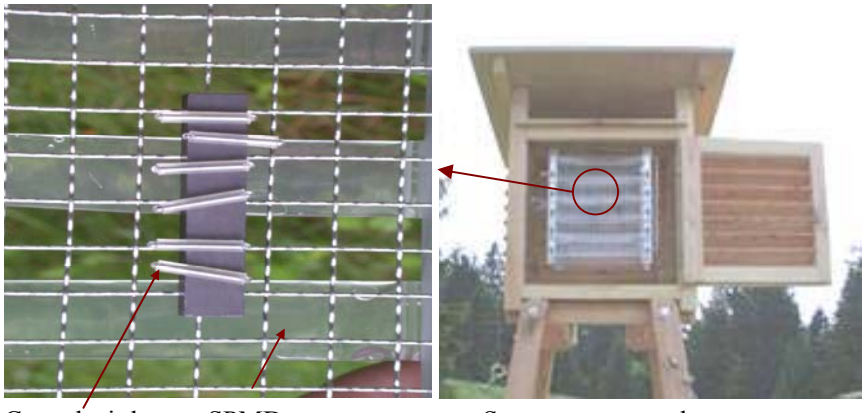


Figure 2-10: Deployment structure for coated stir bars and SPMD.

The passive samplers were transported in clean glass material to and from the place of deployment to avoid contamination. SPMD, without previous cleaning of their surface, and coated stir bars, cleaned with a paper tissue on their surface, were stored in hermetic glass vials and kept under identical storage conditions at -20°C until analysis.

The weight of SPMD was determined before and after the deployment as a quality control of possible triolein losses. SPMD with more than 10 % loss of weight were discarded and a new SPMD sample was analysed. Less than 5 % of the total analysed SPMD had to be discarded in this study.

In addition, active sampling campaigns were conducted in consecutive periods as shown in Table 2-5. The percentages of the different wind directions of the total air pumped for each sampling campaign are also given.

Table 2-5: Active sampling campaigns at Haidel: sampling periods and predominant wind directions.

Period	*Campaign codes*	*NE (%)*	*SE (%)*	*SW (%)*	*NW (%)*	*Calm (%)*	*Time (days)*
20.08.03 -16.10.03	**A**	13.0	11.0	21.1	11.4	43.4	56
16.10.03 -27.01.04	**B**	13.6	17.1	17.1	13.8	38.3	100
27.01.04 -26.03.04	**C**	12.0	12.4	21.3	22.8	31.4	57
07.09.04 -26.10.04	**E**	7.9	13.8	19.0	16.7	42.6	50
26.10.04 -02.02.05	**F**	8.4	12.8	23.2	21.2	34.4	98
25.08.05 -10.11.05	**I**	13.8	26.6	11.7	9.6	38.3	78
10.11.05 -17.03.06	**K**	11.8	9.4	17.0	13.8	48.0	125
17.03.06 -11.07.06	**L**	7.3	9.7	16.6	15.0	51.4	112

Campaigns D, G and H were lost. Note that the cartridges from campaigns A and E were collected at the same time as coated stir bars, SPMD and needles. In the following chart the active sampling campaigns, SPMD and coated stir bars deployment periods and needle collection are shown (Figure 2-11).

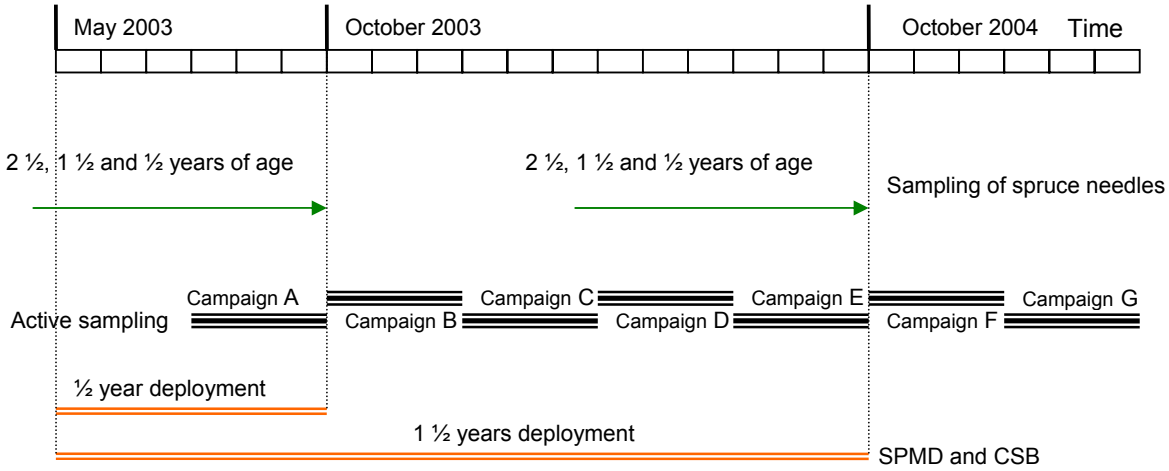


Figure 2-11: Sampling chart at the Bavarian and Bohemian forests.

Humus and mineral soil was sampled in October 2004. Extra campaigns of ½ year SPMD deployment and needle collection were performed in October 2005 and 2006.

2.3.2 Alpine region sites

40 sampling sites (yellow points) were located all along the alpine mountainous chain, out of which 7 (orange points) were altitude profiles (Figure 2-12).

The altitude profiles had 4 to 8 monitoring points at different heights but at the same geographical site. The altitude profiles are coded with the country abbreviation prior to the

sampling site number followed by a subplot number that increases with the altitude. Table 2-6 depicts the codes of the sampling sites of altitude profiles.

More information about the sampling sites is included in the MONARPOP official page: http://www.monarpop.at.

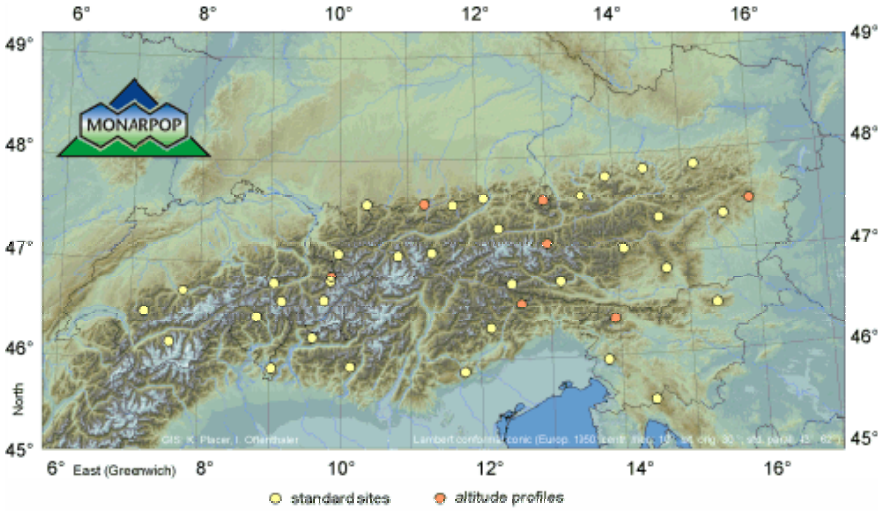


Figure 2-12: Sampling sites in the Alpine mountain region: MONARPOP project.

In general, the sampling sites were at an average altitude of 1400 m a. s. l. and situated in dominant spruce stand sites away from contaminant sources. Needles, humus and mineral soil were collected at all sampling sites. SPMD and low active samplers were employed at the altitude profiles. The sampling procedures were also given in the above mentioned MONARPOP official page, to which the interested reader is referred.

Table 2-6: Altitude profiles in the Alps.

Countries	*Sampling sites: codes and heights (m a.s.l.)*							
Switzerland	CH-01-1	CH-01-2	CH-01-3	CH-01-4	CH-01-5	CH-01-6	CH-01-7	CH-01-8
Height (m a.s.l.)	*1300*	*1410*	*1470*	*1520*	*1600*	*1700*	*2540*	*2663*
Italy	IT-04-1	IT-04-2	IT-04-3	IT-04-4	-	-	-	-
Height (m a.s.l.)	*1123*	*1305*	*1553*	*1656*	-	-	-	-
Germany	DE-21-1	DE-21-2	DE-21-3	DE-21-4	DE-21-5	DE-21-6	-	-
Height (m a.s.l.)	*830*	*1030*	*1230*	*1450*	*1650*	*2650*	-	-
Germany	DE-23-1	DE-23-2	DE-23-3	DE-23-4	-	-	-	-
Height (m a.s.l.)	*805*	*1005*	*1210*	*1420*	-	-	-	-
Slovenia	SI-34-1	SI-34-2	SI-34-3	SI-34-4	-	-	-	-
Height (m a.s.l.)	*1194*	*1354*	*1532*	*1638*	-	-	-	-
Austria	AT-47-1	AT-47-2	AT-47-3	AT-47-4	AT-47-5	AT-47-6	-	-
Height (m a.s.l.)	*1134*	*1381*	*1470*	*1614*	*1779*	*3100*	-	-

In the text, further details about the samplings are presented only when it is necessary for the discussion of results.

2.4 Laboratory Procedures

2.4.1 Analytical determinations

Sample clean-up and extraction techniques were carried out as documented in the form of Standard Operation Procedures (SOPs) in the accredited Dioxin Laboratory at the Institute of Ecological Chemistry in the German Research Center for Environmental Health. The quality assurance system is accredited according to the norms DIN EN ISO/IEC 17025 (Certificate DAC-P-0141-01-00). Due to this, these techniques are not described in detail here but briefly mentioned according to the needs of the present work. The laboratory works with a quality assurance (QA) and a quality control (QC) system related to the analytical determinations for the PCCD/F, PCB, PAH and OCP. This means to accomplish blanks of the extraction and clean-up procedures, to carry out blanks of the analysed matrices, to use standards of reference, to calibrate the instruments and to perform replicates of samples and blanks, among others activities.

2.4.1.1 Clean-up procedure

The extraction and clean-up procedures for PCDD/F and PCB (Figure 2-13) and for PAH and OCP (Figure 2-14) are given schematically as well as a short explanation about the performed steps. To remove interferences, several liquid chromatography steps are carried out consecutively:

PCDD/F and PCB

Sandwich column: Composed by silica gel, silica gel treated with 44 % H_2SO_4 and anhydrous Na_2SO_4. The objective is to eliminate interfering organic compounds by reactions of oxidation and sulfuration with sulfuric acid as an oxidant agent. Na_2SO_4 eliminates possible water remains from the extraction step. This step also removes 2 to 4 ringed PAH.

Reversible carbon column: This step separates the PCB from the PCDD/F. It is called reversible because after a first eluting with *n*-hexane to obtain the PCB fraction, the column is inverted and upside-down eluted again to get the PCCD/F fraction with toluene. This separation is based on the different affinity of PCB and PCDD/F for *n*-hexane.

Solid Phase Extraction (SPE) column: The octadecyl (C18) silica gel is composed of C18 hydrocarbon bounded to the silica gel support. This column retains in the hydrophobic stationary phase long chain hydrocarbons that can interfere in the posterior analysis.

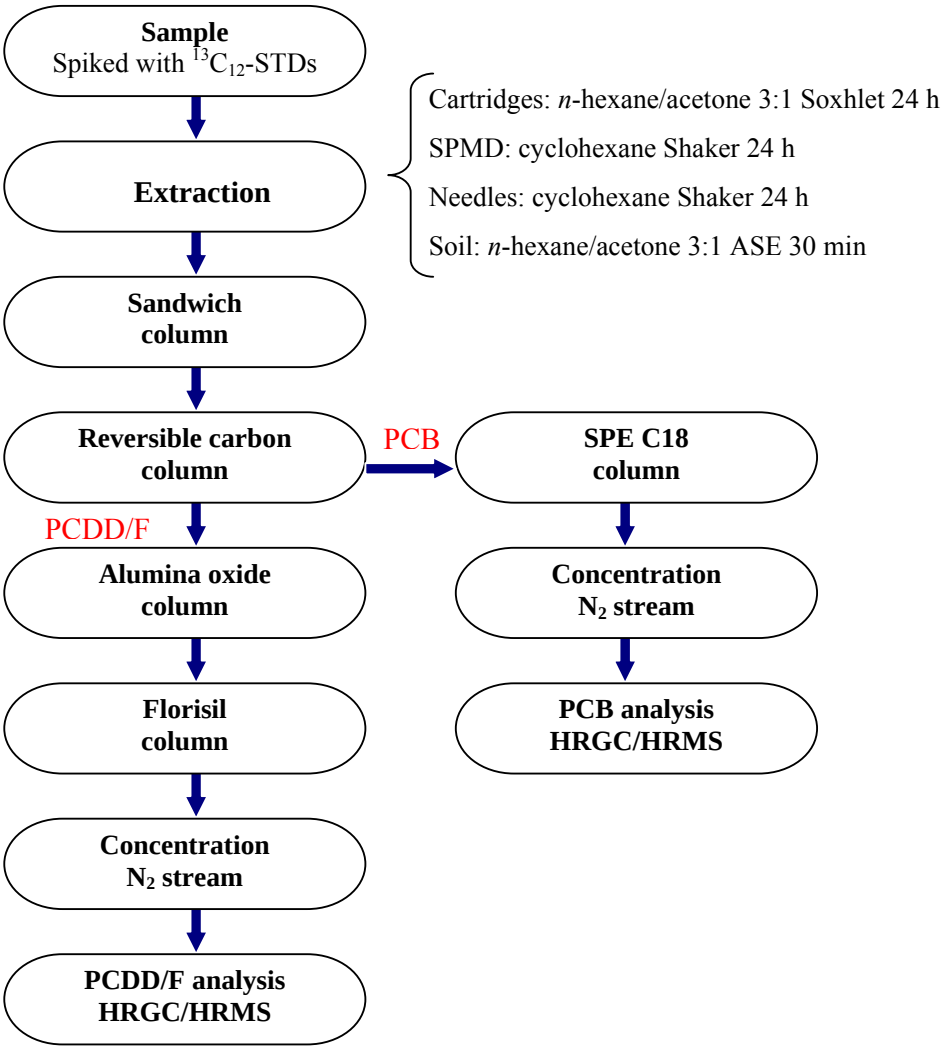


Figure 2-13: Sample preparation scheme for PCDD/F and PCB. ASE: Accelerated Solvent Extraction.

Alumina oxide column: This step is carried out according to Thielen and Olsen (1988). The basic alumina oxide removes non-polar halogenated aromatics, sulphur compounds and polar pollutants, among others.

Florisil column: Florisil is magnesium silicate with basic properties. This column eliminates chlorinated aromatic compounds based on the selection of the correct elution solvent, in this case, dichloromethane.

PAH and OCP

Mixed column: Composed of an alumina oxide layer and a silica gel layer. This column eliminates non-polar halogenated aromatics such as PCB and polar contaminants. The silica layer removes also polar pollutants as well as lipids.

The **SPE column** was already described for PCDD/F and PCB.

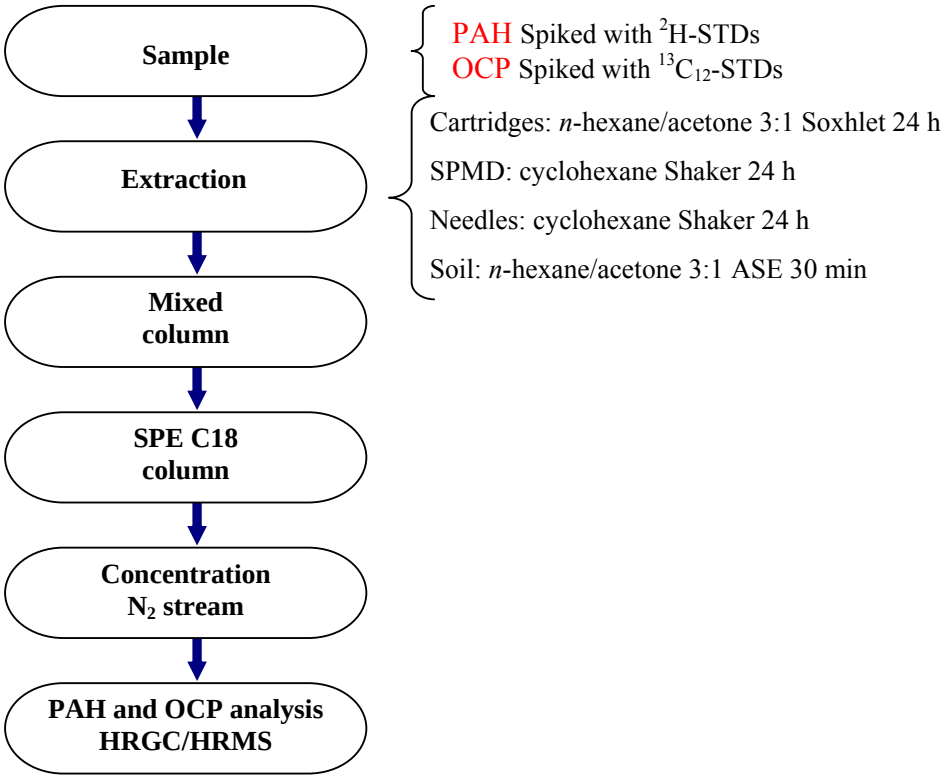


Figure 2-14: Sample preparation scheme for PAH and OCP.

2.4.1.2 Detection and quantification of POP

The analytical determinations of POP are performed by a high-resolution mass spectrometer (HRMS) Finnigan MAT 95 (Thermo Electron GmbH, Bremen, Germany) coupled with a high resolution gas chromatograph Agilent GC 5890 (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separation is achieved by splitless injection (cold injection system CIS3, Gerstel GmbH, Mülheim, Germany) on different columns (Restek GmbH, Sulzbach, Germany) depending on the family of compounds analysed. The mass spectrometer is operated in select ion monitoring (SIM) mode at a resolution of 8000. The two most intense ions of the molecular ion cluster are monitored for the unlabelled and labelled standard isomers. The ^{13}C-labelled standards and the deuterated PAH standard mixtures are purchased from LGC Promochem (Wesel, Germany).

In the case of coated stir bars, POP are desorbed by a thermal desorption unit which is connected on line with the HRGC/HRMS. This newly developed method is detailed in Henkelmann *et al*. (2007).

For the quantification of the analytical results the following parameters have to be considered (Henkelmann *et al*., 1996; Oheme, 1998): a) Response factors between the recovery and internal standards in order to determine the recovery rates for each analysis (recovery rate in the range of 70-115 %). b) Response ratio between native and the equivalent labelled compounds (ratio 1 ± 10 %). If these parameters are satisfactory, the laboratory procedures were carried out successfully.

After this first check, the analysis results are quantified considering the next points: a) Retention time differences between the analyte and its corresponding standard has to be less than 3 seconds. b) The measured isotope ratio should not be apart more than ± 15 % of the theoretical value c) The signal/noise ratio has to be at least in a 3:1 relation. If these considerations are accomplished, the analytical measurements are adequate, allowing the quantification of the analysed compounds.

2.4.2 Bioassay determinations

As the analytical determinations study each compound individually, they are not representative of the environmental responses generated by the whole sample. Assays based on a determined receptor are able to detect all compounds in the sample that exhibit response to it (Müller, 2002). Due to this, two bioassays considering two different potential effects are conducted. One is the EROD micro assay used to detect AhR inducers, which are related to the potential teratogenicity and carcinogenicity of the sample. The other is a yeast estrogen bioassay to determine estrogenic inducers able to cause functional disorders in reproduction, development and cell differentiation, among others. In this section, the background and methods of these two bioassays are explained. The bioassays are carried out in the Laboratory of Ecotoxicology (security level S1) at the Institute of Ecological Chemistry in the German Research Center for Environmental Health.

2.4.2.1 EROD micro assay

Proteins such as some of the P450 enzymes are substrate inducible. This process is a mechanism of protection to eliminate xenobiotics that otherwise would accumulate in the organism. On the other hand, the enzyme induction for one substrate can cause the increased metabolism of another existing enzyme (Whitlock, 1999) generating secondary effects. Our enzyme of interest is the cytochrome P4501A1. The Aryl hydrocarbon Receptor (AhR) mediates the agonist response having high binding affinity and broad substrate specificity. This receptor is found in the cytoplasm together with the protein hp90. The receptor binds to

the xenobiotic liberating hp90. The ligand xenobiotic-receptor induces a structural change and the dimer is able to translocate to the nucleus. There, the AhR connects with the Aryl receptor nuclear translocator (Arnt) structurally through bHLH-PAS (basic helix-loop-helix/Per-Arnt-Sim) proteins forming a heterodimer that acts as a transcription factor for the CYP1A1 gene. This transcription is AhR and Arnt-dependent, but the promoter is activated only in presence of the heterodimer. When all these steps take place (Figure 2-15), the transcription starts generating the ribosomal P4501A1.

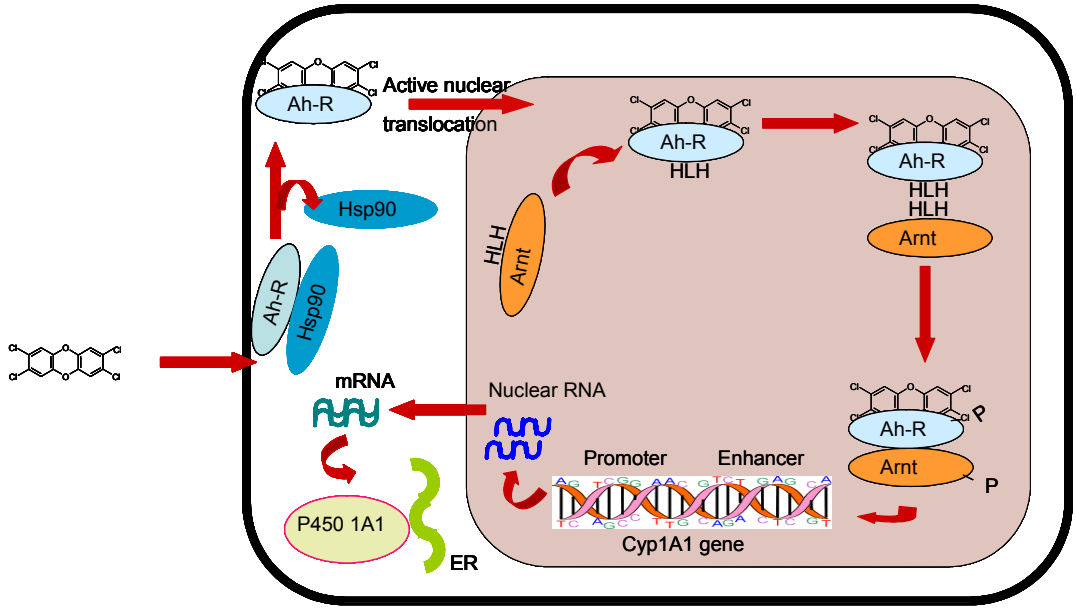


Figure 2-15: Schematic representation of Cytochrome P4501A1 induction (modified from Whitlock *et al*., 1996).

The cytochrome P450 is used for the bioassay due to its high sensitivity and simplicity and because the AhR activation can be indirectly measured by 7-ethoxyresorufin-*O*-deethylase (EROD) activity. The reagent 7-ethoxyresorufin added after induction losses an ethyl group in the presence of cytochrome P4501A1 (Figure 2-16). This deethylation produces Resorufin that is quantitatively measured by fluorescence.

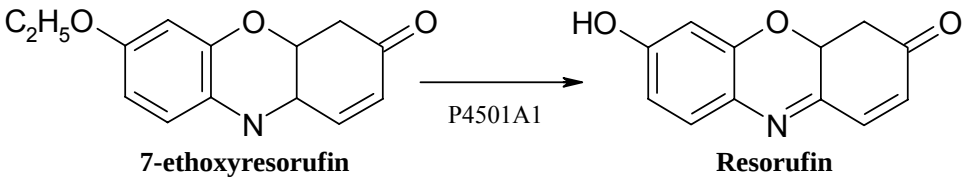


Figure 2-16: 7-ethoxyresorufin-*O*-deethylation reaction.

TCDD, a potent inductor, is used as the xenobiotic of reference in this bioassay. Planar aromatic compounds or compounds close to planarity such as PAH, PCB and PCDD/F are chemicals able to produce a response in this bioassay.

2.4.2.2 Extraction of bio-accumulative compounds

The samples were subjected to a pre-treatment, which selected organic bio-accumulative compounds from the complex mixture. Samples were Soxhlet extracted with 800 ml toluene for 24 hrs in order to obtain the lipophilic extracts.

2.4.2.3 Clean-up to select persistent compounds

The obtained extract was concentrated to 4 ml and then a clean-up step was carried out on a "sandwich" column. The finality of this column is to eliminate the non persistent compounds and organic interferences that can either generate cytotoxicity in the EROD bioassay or are non persistent and polar after treatment with sulphuric acid (Schwirzer *et al.*, 1998).
The column consisted of 10 g active silica gel, 20 g silica gel (44 % H_2SO_4), 40 g silica gel (4 % H_2O), 10 g Na_2SO_4 from bottom to top. The sample was eluted with 900 ml n-hexane. The eluate was concentrated and transferred to 200 µl DMSO under N_2 stream at 45 °C. The carrier solvent for the bioassay was a mixture of DMSO/ Isopropanol 4:1 where persistent bio-accumulative compounds are present.

2.4.2.4 EROD micro bioassay procedure

The rat liver cell line (HII4E) expressing the cytochrome P450 is cultured to perform the ethoxyresorufin-*O*-deethylase (EROD) bioassay based on Donato *et al*. (1993), with modifications as described in Schwirzer *et al*. (1998). In brief, H4IIE cells are incubated in culture flasks at 37°C and 7.5 % CO_2. The hepatic cells are trypsinised and then counted (Neubauer cell counter) and diluted in supplemented medium (DMEM) in order to achieve a cell concentration of $1x10^5$ cells ml^{-1}. This H4IIE cell suspension is seeded into 96 well plates and incubated for 24 hours. Following this, the cells are exposed to 2,3,7,8 TCDD standard concentrations and to the sample extracts for 24 and 72 hours. Blanks are also carried out. After incubation, the medium is substituted by a 7-Ethoxyresorufin solution 8 µM and the Resorufin produced is measured by fluorescence (excitation 535 nm, emission 590 nm) after 30 minutes incubation. The protein determination is performed according to Pierce (Micro BCA Protein Assay Kit). The EROD induction of the sample after incubation is compared to the 2,3,7,8-TCDD response. The obtained values are interpolated in a dose-response function

regarding TCDD induction. This four parameter logistic function is described by the minimum value A equal to the limit of quantification, the slope of the curve B, the EC_{50} value C and the asymptotic value D representing the ligand efficiency or maximal induction. EC_{50} (Effect Concentration 50 %) is the concentration that induces a response half of the maximal possible.

$$y = D + \frac{A - D}{1 + \left(\frac{X}{C}\right)^{B}}$$ 4 parameter logistic function

The results are given as toxicity equivalent values (TE values) pg TCDD per g sample. TE values obtained after 72 hours incubation are related to the persistent compounds in the sample. TE values obtained after 24 hours incubation are related to all compounds able to elicit response in the bioassay, thus persistent and non-persistent. In the non-persistent group are found high molecular weight PAH not eliminated in the clean-up step. TE values are used to compare toxicities related to a determined mechanism of action. In this case the TE scale compares compounds and their ability to bind to the AhR. Therefore, the implied substances are all the dioxin-like compounds, or compounds with planar aromatic structures able to bind to the AhR.

The bioassay is performed with extracts from the matrices: humus, mineral soil, needles and SPMD. In needles and SPMD, the response values were below the limit of quantification. Possible toxicity effects generated by these matrices in the hepatic cell cultures were controlled performing the resazurin test. This cytotoxicity determination is based on the ability of intact cells to reduce the resazurin reagent (blue) to resorufin (red). After the EROD assay the well plates are washed and incubated with resazurin 0.01 % for 1 hour (Schwirzer *et al*., 1998). The reduction achieved after incubation is detected by fluorescence in a multi-well plate reader (LumiCount, SPECTRA Fluor) excitation 550 nm, emission 590 nm. Higher fluorescence measurement means more intact cells. The fluorescence of the sample is compared with the fluorescence obtained with untreated cells used as a control sample (Strotmann *et al*., 1993).

2.4.2.5 Yeast based estrogenic assay

Estrogenic disrupters are substances that interfere in the structure or function of the endocrine system and thus, in the hormone regulation machinery. The cytochrome involved in the

hormonal regulation mechanisms tested in the yeast based estrogenic assay is also a protein of the P450 super family, as the cytochrome involved in the mechanism of xenobiotic elimination tested in the EROD micro assay. For the yeast based estrogenic assay, the P450 aromatase is the result of the CYP19 gene expression (Simpson *et al*., 1994). This enzyme is called aromatase because it creates a phenolic A-ring from 3 ketone A-ring androgens (aromatization), converting them to the corresponding estrogens. This transformation controls androgen-estrogen levels in the organism. A hormone misbalance causes disorders at the structural and functional level in reproduction and development such as, among others, energy homeostasis (Simpson *et al*., 2005). Estrogens and compounds that mimic estrogens, like PCB 153, dioxins, dieldrin and p,p´-DDT are able to elicit an estrogenic response (Andersson *et al*., 1999, Blair *et al*., 2000). The mechanism of action is complex and will only be described in general terms. The ligand binds to the ER, dimerizes and forms a protein complex with an estrogen responsive element (Shibata *et al*., 1997). This responsive element, which belongs to the gene promoter regions, modulates the gene transcription. This mechanism of receptor-based selectivity allows different tissues producing the same hormones to activate the gene transcription only under the response of selective receptors (Katzenellenbogen *et al*., 1996). The co-activators of the ER, specific of each cellular type, activate the structural changes in the chromatin (Müller *et al.*, 2002). After chromatin re-modelling, mRNA codes are transcripted specifically for one protein. This protein later generates an estrogenic effect in the target issue.

For the yeast based estrogenic assay, a yeast strain (*Saccharomyces cerevisiae*) expressing the human estrogen receptor α (hER α) and the enhanced green fluorescent protein (yEGFP) was used as it is able to generate a response to estrogen exposure. This yeast strain has integrated the hER α reporter but also the yEGFP reporter in its genome (Bovee *et al*., 2004a), instead of the extra-chromosomal expression plasmid presented in other strains (Routledge and Sumpter, 1996). In this way, there is no risk of losing the response of the cell due to the plasmid elimination. Other advantages are lower incubation time and no need to substrate addition. However, it is important to remark that the bioassay sensitivity decreases with this new strain in comparison to the sensitivity achieved with the yeast genetically modified by Routledge and Sumpter (EC_{50}≈0.6 instead of EC_{50}≈0.2). The use of a yeast strain is convenient due to its robustness, low cost and lack of endogenous receptors (Breithofer *et al*., 1998, Bovee *et al*., 2004b). The substance used to perform the dose-response curve is 17-ß Estradiol due to its potent estrogenic activity (Routledge and Sumpter, 1997).

2.4.2.6 Estrogenic bioassay

Extraction and clean-up to select estrogenic substances

Active substances are Soxhlet extracted from the soil with CH_2Cl_2 for 24 hours. After this, the sample is concentrated and filtered through Na_2SO_4 to eliminate possible water remains. Following this, a solid phase extraction (SPE) to eliminate impurities such as linear hydrocarbon chains is conducted. The eluate is concentrated in a N_2 stream and this enriched extract is transferred to 200 µl Methanol as final carrier.

Bioassay procedure

The modified yeast strain was a gift from Bovee T. (RIKILT, Institute of Food Safety, Wageningen University and Research Center, The Netherlands), and the culturing conditions were followed according to Bovee *et al*. (2004a). The bioassay was performed as described in Bovee *et al*. (2004b; 2005), with some slightly modifications. In brief, aliquots of the bioassay medium were treated in parallel with 17ß-Estradiol standards and samples in order to keep the ratio standard/medium and sample/medium at 0.005. The carrier solvent for the 17ß-Estradiol standards was methanol and the standard curve ranged from $5x10^{-12}$ to $2x10^{-9}$ M. 96 well plates, containing standards and samples, were incubated at 30°C, 225 rpm for 15 hours or until reaching the maximal answer in the 17ß-Estradiol standard curve. Solvent blanks with bioassay medium were also included in all 96 well plates. Fluorescence was measured at 535 nm (excitation 485 nm) in a luminescence multi-well plate reader (LumiCount, SPECTRA Fluor). The fluorescence signals were corrected with the blank medium values obtained at the measurement time. Samples, standards and blanks were performed in quadruplicates. From these quadruplicates, the mean value of the 3 with lower standard deviation among the 4 responses was taken as the final bioassay response. The corresponding estrogenic values were calculated using the 17ß-Estradiol dose-response curve (4 parameter logistic function).

Validation: Inter-laboratory

This bioassay was implemented in the Laboratory of Ecotoxicology at the Institute of Ecological Chemistry while this work was in progress. Thus, the quality and standardization of the new bioassay had to be controlled. To achieve this, the laboratory participated in the inter-laboratory "Screening Methods for Water Data Information in Support of the Implementation of the Water Framework Directive (SWIFT-WFD)" supported by the European Union. 6 batches of 11 unknown samples each were screened consecutively. Results were calculated and sent for evaluation by the organizing commission. 7 laboratories from 5 European countries took part in this inter-laboratory. The agreement between

analytical and bioassay results was also determined as a control by the inter-laboratory commission. The analytical determination is described in Días-Cruz *et al*. (2003) and the clean-up and extraction of the samples was performed according to Céspedes *et al*. (2004). The values obtained at the laboratory of Ecotoxicology at the German Research Center for Environmental Health proved to be accurate. The inter-laboratory results were reported internally in the SWIFT deliverable D23: Inter-comparison of estrogenicity in water determined by yeast-based bioassays. More information is given in Section 3.7.2.

Validation: Comparison of two measurement methods

As a second quality step, the bioassay response was measured by two methods: fluorescence at the plate reader (classical method) and by flow cytometry. The fluorescence measurement gives the response per well, so homogeneity and the same cell amount per well are necessary to achieve accuracy and comparable measurements among wells. The second measurement gives a specific fluorescence answer that represents the response per amount of counted cells. The flow cytometry measurements were carried out at the Institute of Toxicology by Dr. Wolfgang Beisker in the German Research Center for Environmental Health.

With this measurement, it is also possible to differentiate between inducted and non-inducted cells. In this way, the capability of response of the yeast strain can be assessed. To achieve this, three substances known for their strong binding affinity (17ß-Estradiol, Diethylstilbestrol and 17α-Ethynylestradiol) were evaluated at different induction doses for the two measurement techniques (Figure 2-17). Diethylstilbestrol is a teratogenicity synthetic estrogen and 17α-ethynylestradiol is a potent endocrine modulator used broadly as an oral contraceptive. In Section 3.7.3, the obtained measurements are analyzed.

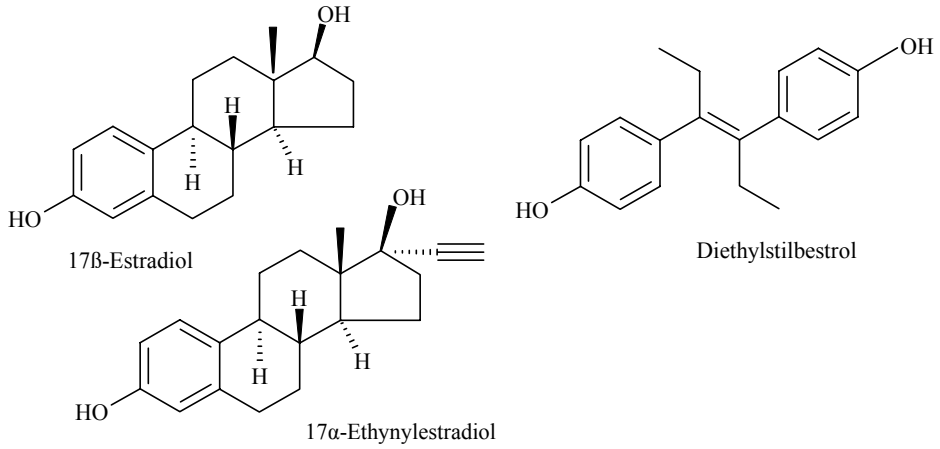


Figure 2-17: Molecular structures of 17 ß-Estradiol, Diethylstilbestrol and 17α-Ethynylestradiol.

2.5 Statistical analysis

The statistical procedure is based on Principal Component Analysis (PCA) created by Karl Pearson (1901) and later developed by Harold Hotelling in 1933. This method analyses and identifies the predominant patterns in a multivariate data matrix. The variation in the data descriptors is projected and correlated in an orthogonal system taking into account the principal components (PCs) of the matrix. In other words, the samples are distributed in a graphic where the origin is the zero variance among them and the dispersion among axes indicates the tendency of each sample related to the others. In this way, the number of variables is reduced allowing the pattern visualization in a graphic with PC axes. The first principal component explains the largest variation within the matrix followed by the second principal component and the third successively. To determine general patterns, it is recommendable that the first and second PCs explain more than 60 % of the total sample variance.

Before performing PCA, non-detectable concentration values were substituted for the limit of detection (LOD) values for each chemical in the sample. The normal distribution of each variable is checked using the Kolmogorov-Smirnov test of composite normality. (Null hypothesis: data normally distributed p-values ≥ 0.5). Variables that failed the test were discarded from the data matrix. The importance of working with normalized data is to avoid results affected by the different orders of magnitude in sample concentrations.

A dendogram describing pattern similarities was also created using the unweighted pair group method with mathematical averages (UPGMA).

The software used for statistical analysis was S-Plus 6.2 and the Multi-Variate Statistical Package (MVSP) version 3.11 (Kovach Computing Services, 2000).

3. RESULTS AND DISCUSSION

3.1 OCP in the Bavarian and Bohemian forests

3.1.1 Air concentrations

All compounds were detected in the active sampler, except for mirex, methoxychlor, ε-HCH and trans-HCE. Correlations between the compounds showed relationships within each family. PeCB and HCB correlated with r^2 =0.95. PeCB is an impurity in the HCB industrial production and formation. Furthermore, HCB degrades as an intermediate compound to PeCB by dechlorination which could be the reason for the high correlation between them in air besides other matrices. The PeCB concentrations approximately amounted to half of the HCB concentrations in all the sampling periods, averaging 33 pg m^{-3} for PeCB and 67 pg m^{-3} for HCB. This is concordant with continental average concentrations for these two compounds (PeCB 45 pg m^{-3} and HCB 89 pg m^{-3}) obtained by Shen *et al.* (2005) in the North American atmosphere. The compound HCB was characterized by the highest air concentrations within this group of chemicals.

α-HCH and γ-HCH were well correlated (r^2 =0.90). The α- and γ- isomers were present in concentrations 50 and 100 times higher than the β-HCH, respectively. δ-HCH was not always detected or detected close to the limit of detection with values around 0.15 pg m^{-3}. The dominant γ-HCH isomer varied between 11 and 34 pg m^{-3} without clear seasonal tendency but higher seasonal variability than α-HCH. This trend could also be observed in other studies (Motelay-Massei *et al*., 2005).

Additionally, correlations among chemical families, namely HCH isomers and cyclodiene pesticides were also found: α- and γ-HCH with cis-HCE (r^2=0.87 and r^2=0.94, respectively). Dieldrin and α-Endosulfan correlated with r^2=0.86. The correlations among compounds of different families could be related to the origin of the contamination. Long-range transport contaminants coming along with the air mass fluxes cause the fluctuations for compounds of similar physicochemical properties to correlate well. Endosulfan is an insecticide and timber preservative of common use in the Mediterranean area and thus showed also high concentrations in the air. In particular, α-Endosulfan with an average value of 28 pg m^{-3} showed high fluctuations between campaigns from 8.6 to 43.4 pg m^{-3}. Endosulfan is employed seasonally during spring time. Thus, concentration variations caused by its episodic application are expected.

There is a tendency to higher DDE concentrations related to DDT concentrations in the colder seasons, which was already statistically observed in a German atmospheric study (Wenzel *et al*., 2006).

Principal Component Analysis was performed with the pesticides detected above the limit of determination for all the sampling campaigns in all wind directions. Figure 3-1 shows higher concentrations in the winter campaign C and the autumn periods A and E at Haidel (A, C and E values in all wind directions placed on the right of the figure).

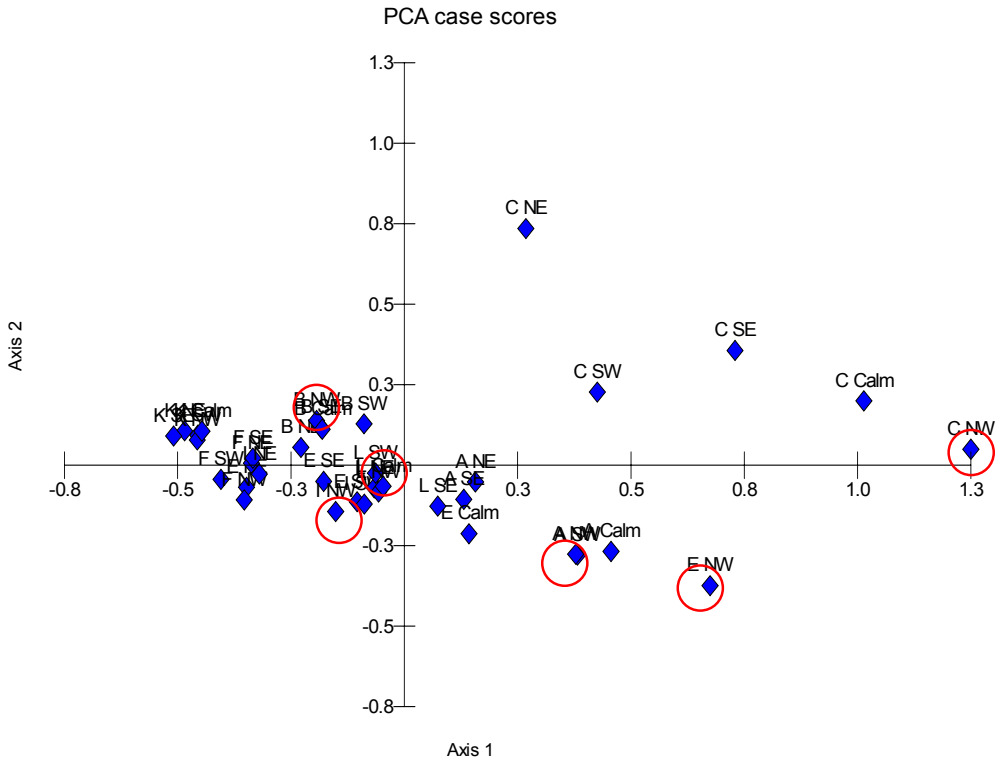


Figure 3-1: PCA case scores (37) of OCP (11) for the active sampling campaigns A, B, C, E, F, I, K and L. Axis 1 accounts for 63.6 % and axis 2 for 13.7 % of the variability (77.3 % of the total).

As depicted by red circles in figure 3-1, the North-West (NW) direction tends to be the wind direction located more to the right for each sampling campaign. A tendency of higher values at this wind direction indicates a major loading when the predominant winds are coming from the North-West direction.

3.1.2 SPMD

SPMD sequestered the same compounds detected in the active air sampling. When comparing the distribution of isomers in SPMD and air, changes in the relationship among isomers were found. For instance, the concentration ratio for HCB/PeCB was 18 in SPMD after 6 months

exposure, whereas this ratio was around 2 in cartridges of the active sampling. The different compound distribution obtained in the active and passive samplers point out the uptake "selectivity" of the SPMD whereas the active sampler reflects the air concentrations. The passive sampler sequestered more HCB than PeCB, the first with a K_{oa} value of one order of magnitude higher than PeCB (8.09 and 7.19, respectively). Furthermore, the HCH isomers also exhibited changes in their concentration distribution. The highest concentrations were detected for the γ-HCH isomer. γ-HCH accumulated 3 times more than α-HCH in SPMD of ½ year deployment, whereas it was only two times more for active sampling. Additionally, the ratio γ-HCH/ β-HCH averaged 24. Taking into account that β-HCH is 100 times less concentrated in air than γ-HCH, β-HCH has a better uptake by the SPMD than the γ-HCH isomer. HCH isomers represent different hydrophilic characteristics, β-HCH being much more hydrophobic than α- and γ-isomers (Quintero *et al*., 2005). This is indicated by the K_{oa} of this compounds being, 8.39, 10.06 and 8.67 for the α-, β- and γ-isomers, respectively. As a consequence, this explains the higher SPMD uptake of β-HCH compared to α,γ-HCH isomers, besides other factors such as the absence of axial chlorines. It is also worth to mention that β-HCH does not only present a better uptake in SPMD but that the bio concentration factor (BCF) in human fat for β-HCH is also similar, being more than 25 times higher than the BCF for the α,γ-HCH isomers (Willett *et al*., 1998). This is concordant with the fact that SPMD mimic biological membranes.

Analysing the compound uptake after 1 ½ years, a change in the relationship between compounds can be observed again. Not only the compound lipophilicity but also the exposure time is a key factor for the uptake kinetics. Figure 3-2 shows the concentrations for the two different exposure periods. Aldrin and HC were close to the limit of detection after 1/2 year exposure, whereas after 1 ½ years exposure, low concentrations were detected in SPMD. For these compounds exhibited in low air concentrations long deployment periods are advisable for proper detection and monitoring. In contrast, PeCB and HCB decreased in their SPMD concentrations after the longer exposure period. The concentrations in air for PeCB and HCB were 26.4 and 65.8 pg m^{-3} in the campaign A (coincident with the end of ½ year SPMD deployment) decreasing to 24.0 and 40.3 pg m^{-3} in the campaign E (coincident with the end of 1 ½ year SPMD deployment), respectively. Therefore, long deployments to determine PeCB and HCB are not advisable if a linear integrative uptake is desiderable. As a consequence, in order to determine the adequate deployment period and thus, real air concentrations using SPMD, it is necessary to know the current uptake stage for each compound (Bartkow *et al*., 2005).

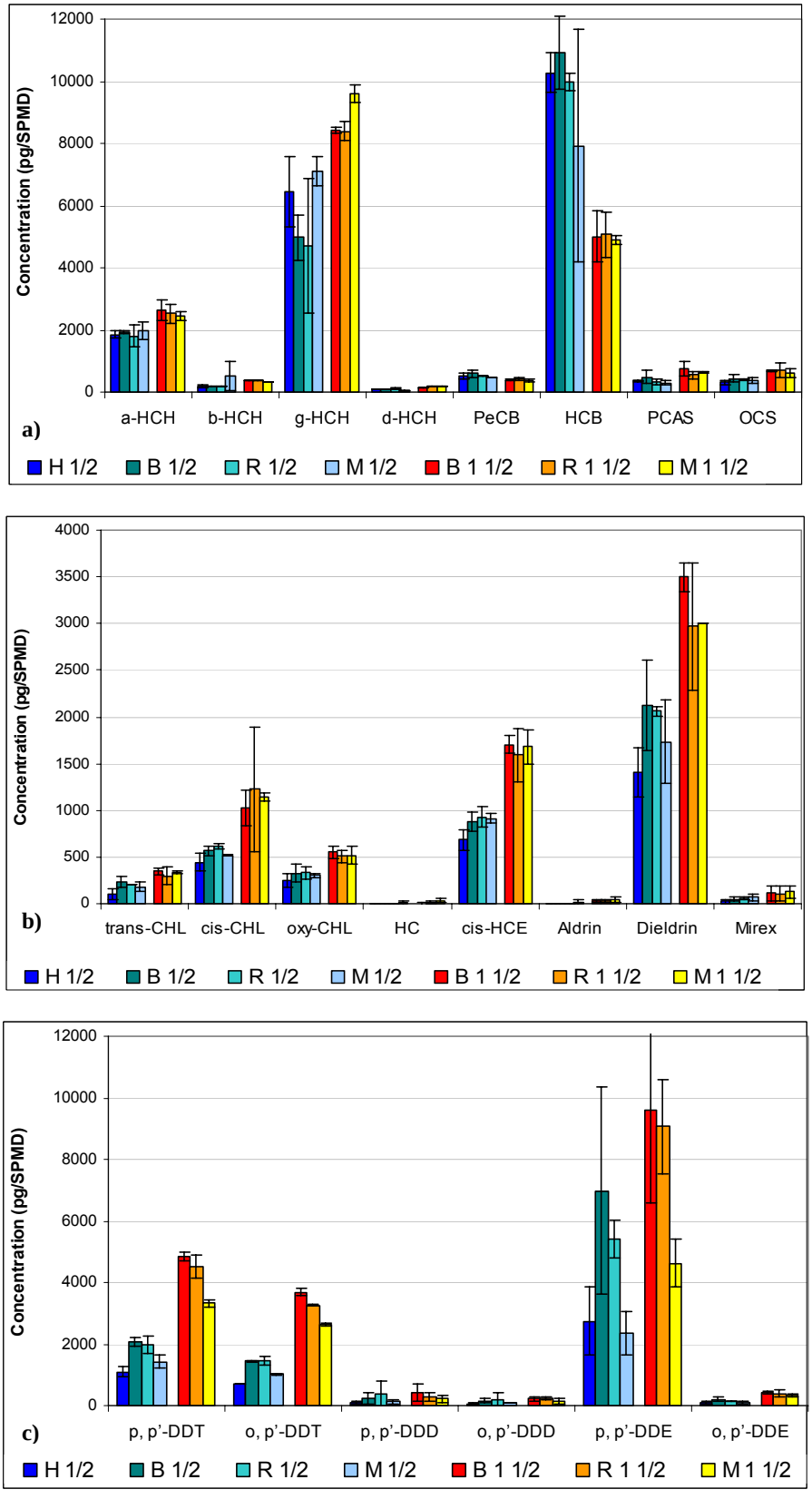


Figure 3-2: Concentrations in SPMD after ½ year at 4 sites (n=2) and 1 ½ years (n=2) exposure at 3 sites. a) HCH isomers, OCA, PCAS, PeCB and HCB b) Chlorinated pesticides c) DDT family. H: Haidel, B: Boubin, R: Ruckowitzschachten and M: Mitterfels. ½ : ½ year deployment 1 ½: 1 ½ year deployment.

One of the compounds found at higher concentrations in the SPMD with longer exposure was α-endosulfan. The concentration increased from 39.4 ng in SPMD exposed ½ year to 61.9 ng per SPMD for 1 ½ years of exposure. As well, ß-endosulfan increased on average from 5.6 to 14.4 ng per SPMD during the extended exposure time. The ratio between these two isomers also changed significantly with exposure time. The ratio α/ß was on average 7 for the ½ and 4 for the 1 ½ exposure period. This indicates a relative enrichment of the ß-isomer in comparison to the α-isomer with time that can not be explained by a higher affinity of the ß-isomer for the lipidic phase (K_{oa} 9.81 and 9.68 for the α- and ß-isomer, respectively). One possible factor is the relative higher air concentrations of ß- in comparison to α-isomer in the campaign E than in the campaign A. ß-endosulfan increases from 2.4 to 3.7 pg m^{-3}, whereas α-endosulfan remains in the same concentration level (30.3 and 31.3 pg m^{-3} in campaigns A and E, respectively). Higher sampling rates for ß- than α-endosulfan were obtained in SPMD after ½ year deployment at Haidel (see Table 3-17), but this was not observed in other sampling sites under study (Table 3-16).

The concentrations in active air cartridges and SPMD ½ year deployment correlated well (Figure 3-3), in particular for DDT and their derivatives.

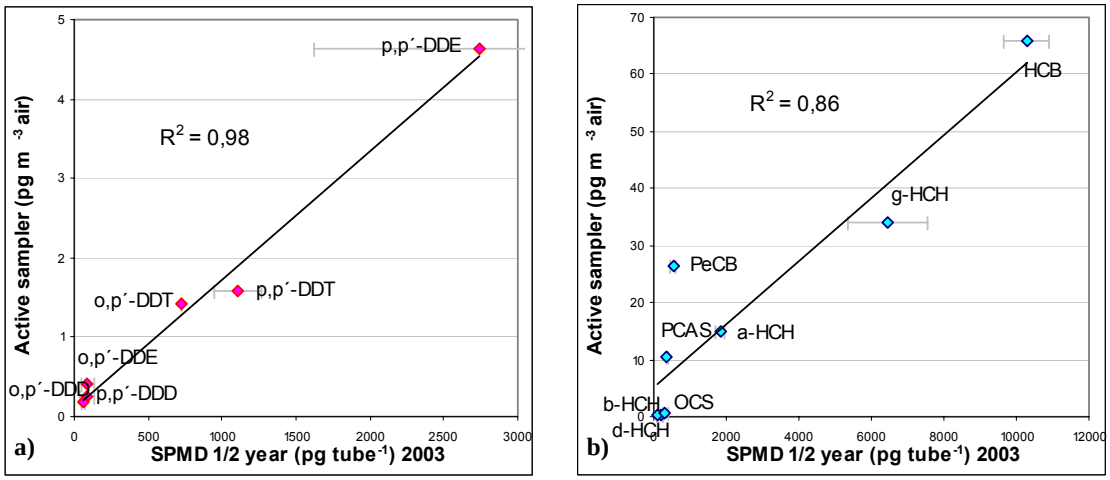


Figure 3-3: Correlations of SPMD ½ year exposure (n=2) and 3 months active sampling at Haidel. a) DDT family b) HCH isomers, OCS, PCAS, PeCB and HCB. Sampling October 2003.

Good correlations between air concentrations and SPMD ½ year exposure for the DDT family were also obtained in the sampling campaigns, 2005 and 2006 (Figure 3-4). DDD compounds were not always detected by means of active sampling but were sequestered in the SPMD

showing the capacity of integrative uptake of compounds in these passive devices until levels above the LOD which were not achieved with active air samplers.

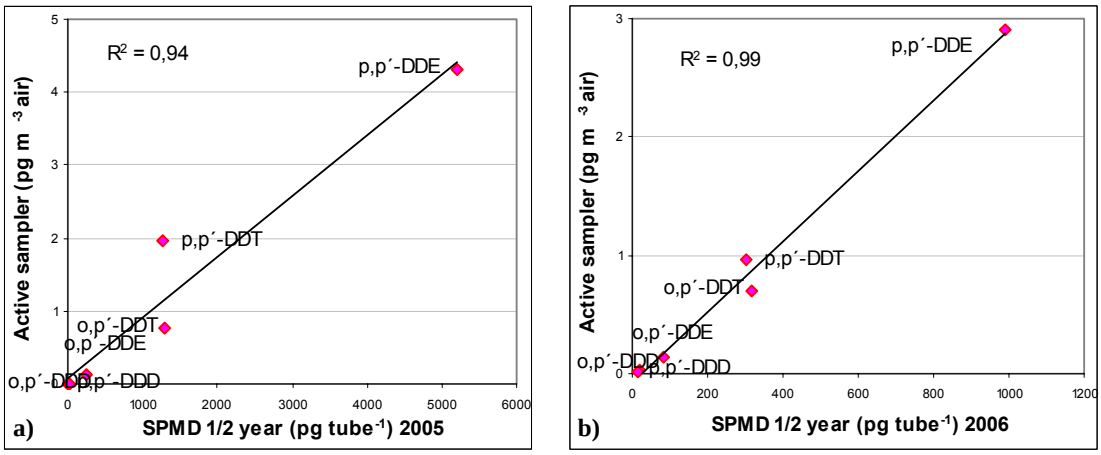


Figure 3-4: Correlations of SPMD ½ year exposure and 3 months active sampling at Haidel. a) Year 2005 b) Year 2006.

3.1.3 Needles

Needles were collected in October 2003 and October 2004 at the four sampling sites. OCS, trans-HCE, mirex and heptachlor were not detectable. However, ε-HCH neither detected in active campaigns nor SPMD, was detected in all needle ages. The general trend to observe is higher compound concentrations in older needles with the predominant compounds γ-HCH, p,p'-DDT, p,p'-DDE, HCB, α- and β-endosulfan. This trend was found in all sampling campaigns as shown here for the HCH family (Figure 3-5). As an example γ-HCH exhibited mean concentrations of 710 pg g^{-1} dry needles for 2 ½ year old needles and 380 pg g^{-1} dry needles for 1 ½ year old needles in 2003. The needles with an age of ½ year were characterized by γ-HCH mean concentrations of 190 pg g^{-1} dry needles in 2003 and 180 pg g^{-1} dry needles in 2004.

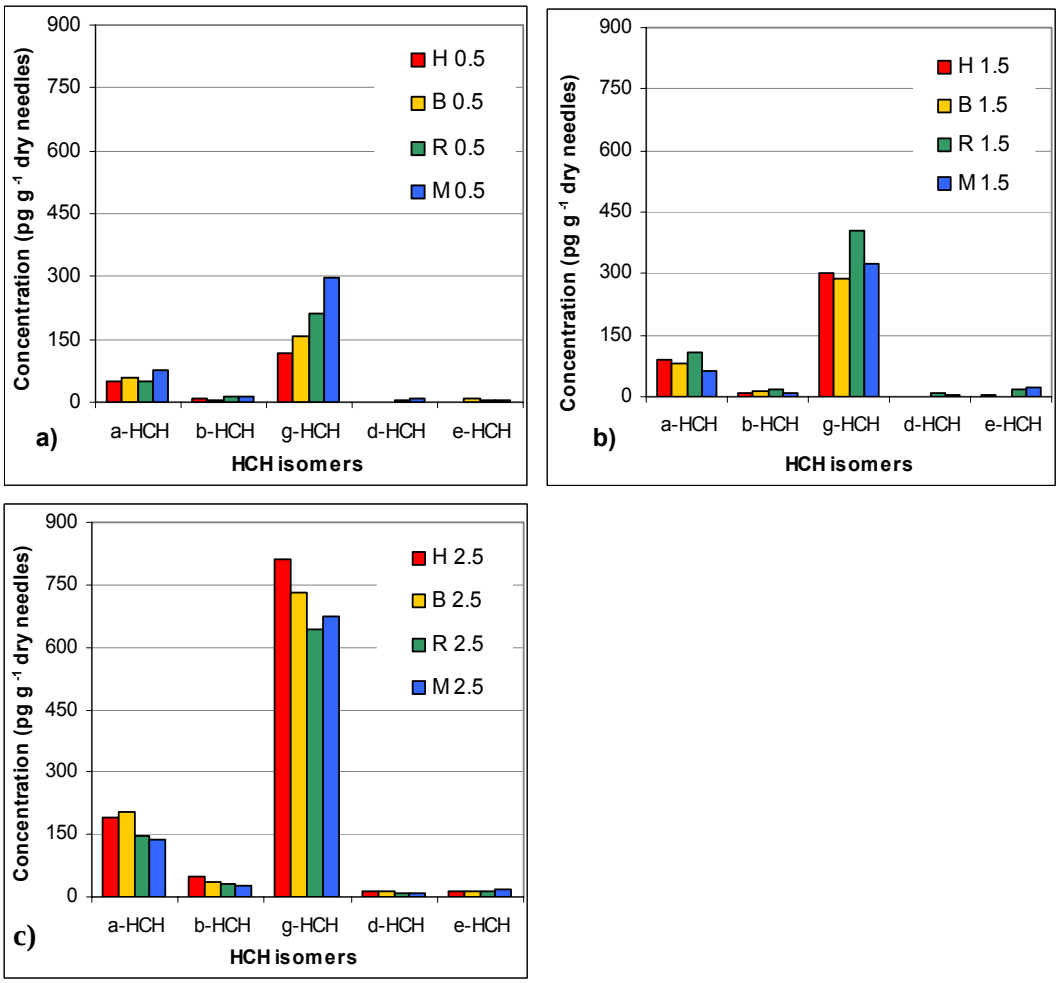


Figure 3-5: HCH isomer concentrations (pg g^{-1} dry weight needles) in a) needles ½ year b) needles 1 ½ years and c) 2 ½ years of age at 4 sampling sites in 2003. H: Haidel, B: Boubin, R: Ruckowitzschachten, M: Mitterfels, 0.5: ½ year, 1.5: 1 ½ years and 2.5: 2 ½ years old needles.

The pattern among compounds was the same in the different years and ½ year old needles in Mitterfels showed a tendency to higher pesticide concentrations when compared to the other sampling sites (Figure 3-6). In contrast, SPMD accumulation at Mitterfels was lower for the DDT family (Figure 3-2c) than at the other sampling sites. DDT distributed in the atmosphere at continental scale, exhibit different concentrations at these remote sampling sites (even to 5 times in concentration as seen in figure 3-6). If it is assumed no recent use of DDT, these concentration differences indicate the influence of the local conditions in the needle uptake not allowing a "standard needle matrix" among the different sampling sites. However, the recent use of DDT cannot be discarded due to historical facts that indicate the use of DDT at regional level. On the other hand, errors inherent to the laboratory procedures should not be disregarded. In conclusion, sample repetitions are necessary to confirm these findings.

Nevertheless, needles are a useful qualitative tool for detecting atmospheric contaminants and estimating their distribution and patterns.

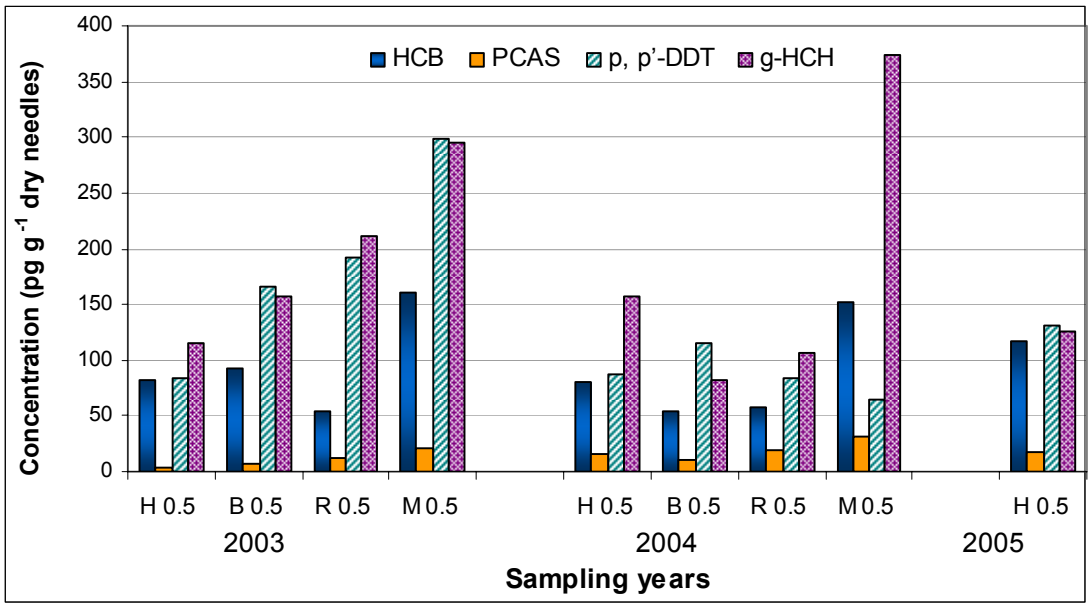


Figure 3-6: HCB, PCAS, p,p´-DDT and γ-HCH concentrations (pg g^{-1} dry needles) in needles ½ year of age at 4 sampling sites. H: Haidel, B: Boubin, R: Ruckowitzschachten, M: Mitterfels.

Organochlorine accumulation in needles did correlate neither with the active sampling nor with SPMD uptakes.

3.1.4 Soil

Comparing concentrations in humus and mineral soil, humus presents a higher accumulation of OCP due to higher amounts of organic matter (Table 3-1). High amounts of organic content are observed in the soil defined as a mineral soil. This is due to the non-classical type of soil sampling described in Section 2.2.3. The mineral soil is defined as the layer sampled after removing the forest humus until 10 cm depth below the humus soil but not beyond this depth, implying in some cases higher amounts of organic matter when not all the mineral soil profile was sampled. This is observed for the B and R mineral soil (samples with high organic content). Organochlorine compounds are hydrophobic and are attracted to the organic matter where they are accumulated. This effect is even more important in forest soils characterised by high amounts of organic matter (Wania and McLachlan, 2001). Due to this, the OCP amounts normalised to the organic content were calculated to achieve a better comparison among sampling sites (Table 3-1).

Boubin and Ruckowitzschachten presented more than twice the pesticide accumulation in their humus layer than in the mineral layer, whereas Haidel and Mitterfels showed 7 and 14 times more, respectively. This is related to the organic carbon content differences.

Table 3-1: Organic carbon content (%), total OCP concentration (pg g^{-1} dry soil) and OCP normalized (ng g^{-1} organic carbon content) in humus and mineral soil at 4 sampling sites.

Sample code	*Soil type*	*Organic carbon content (%)*	*OCP (pg g^{-1})*	*OCP normalized (ng $g^{-1}_{\text{organic carbon content}}$)*
Boubin	mineral	34.5	33656	98
Haidel	mineral	7.6	7222	95
Ruckowitzschachten	mineral	12.1	19467	161
Mitterfels	mineral	7.2	3618	50
Boubin	humus	46.4	72139	155
Haidel	humus	41.4	53827	130
Ruckowitzschachten	humus	30.3	44870	148
Mitterfels	humus	45.0	52763	117

The lower ratio humus/mineral soil due to a higher mean concentration in the mineral layer in Ruckowitzschachten could be seen as an indicator of a higher load of contaminants in the past. In addition, the higher organic content of Boubin in comparison to the other mineral soils is in agreement with the higher accumulations obtained. Differences in soil texture may have had influence on the different ratios obtained for OCP in the mineral and humus soils at Mitterfels (silt loamy and loamy sand textures) in comparison to the other sampling sites (sandy loam and silt loam textures).

All the compounds analysed were detected in the soils, including mirex and trans-HCE, neither detected in needles nor in active campaigns. p,p´-DDT, o,p´-DDT and p,p´-DDE, represented on average more than 74 % of the total pesticides in mineral soil and 67 % in the humus soil. Analysing the DDT compounds in detail, p,p´-DDT was on average 50 % and 60 % of the total DDT family in the mineral and humus soil, respectively. o,p´-DDT remained without significant changes in both types of soil at 12 % of the total DDT compounds. p,p´-DDE, the main degradation product of DDT, was around 37 % (mineral soil) and 27 % (humus soil). o,p´-DDE was only found as a minor component in the mineral soil. Taking into consideration that the technical mixture contains approximately 77 % of p,p´-DDT and 15 % of o,p´-DDT, a decrease in the p,p´-DDT percentage in the soil related to the original mixture was observed (77 % to 50-60 %). This decrease was not so clear for the o,p´-DDT compound which decreased from 15 % (technical mixture) to 12 % (soil). In addition, the ratio ΣDDT/ΣDDE averaged 1.7 in mineral soil and 2.9 in the humus soil. This indicates the higher

DDT depletion in the mineral than in the humus soil. One possible reason for the higher ratio ΣDDT/ΣDDE in the humus soil is the higher DDT concentrations that can auto-inhibit the DDT biodegradation processes (Pereira *et al*., 1996). Other possibility is recent DDT input due to its use as a pesticide in the region. Relatively high amounts of DDT in relation to its metabolites were found in this study in comparison to other studies (Minh *et al*., 2006). The almost negligible amounts of DDD isomers could be related to the type of degradation pathway that DDT undergoes. The formation of DDD metabolites is favoured under anaerobic conditions, and DDD is almost not present in the superficial oxygenated organic layer and exhibit low concentrations in the mineral soil (Heberer and Dünnbier, 1999). On the other hand, it has to be considered that DDD metabolites are not as persistent as DDE metabolites under aerobic conditions.

Despite the ban on DDT since the end of the 1980s in Western Germany, DDT values are considerably high. DDT mean values in mountainous agricultural areas in China are ΣDDT 9.46 ng g^{-1}dry weight (Wang *et al*., 2006) and with long term DDT usage are ΣDDT 56.0 ng g^{-1}dry weight (Gong *et al*., 2004), whereas in the German-Czech forested areas the mean value in mineral soils is ΣDDT 12.9 ng g^{-1} dry weight. ΣDDT represents the sum of the six metabolites. In the humus soil the mean value is even higher: 118.2 ng g^{-1} dry weight. This can be related to stronger pesticide adsorption to organic particles in this layer and thus less bioavailability. It is important to remark that DDT import was allowed until 2001 in the Czech Republic (PAN Pesticide Database). This could be a possible recent DDT input source to the mountain forest ecosystem, but also long-range atmospheric transport in the past from other areas like the former German Democratic Republic can not be excluded. Studies in the Czech Republic conducted by Kosubová *et al*. (2005), in a forest soil close to the Czech site Boubin showed a ΣDDT concentration of 61.4 ng g^{-1} which agrees to the higher amounts detected in the context of this work in Boubin (ΣDDT 27.7 ng g^{-1} dry weight). Boubin exhibited the highest DDT loadings, followed by Ruckowitzschachten, a site located at the central mountainous crest between Germany and the Czech Republic. The lower loaded sampling sites are located at the western side of the mountainous barrier, on the German side.

The other major components detected in soil were dieldrin, HCB and γ-HCH. γ-HCH decreased on average from 8 % in organic soil to 2 % in mineral soil. In the case of β-HCH, the percentage decrease observed was from 3 % (humus soil) to 1.4 % (mineral soil). Studies in soils have shown a higher biodegradation of α- and γ- isomers than for the β-isomer (Quintero *et al*., 2005). This could explain the similar γ- and β-HCH accumulation in mineral soils in spite of the lower air concentrations of β-HCH. The mean γ-HCH/α-HCH ratio was

1.2 for the mineral soil and 4.0 for the humus soil. These ratios indicate a higher contribution of lindane (γ-HCH) to the total soil concentration than the HCH technical mixture. This is in agreement with the fact that the technical mixture was mainly used initially and later substituted by lindane. Boubin mineral soil and a forest soil close to Sumava Park presented ΣHCH values of 1.2 and 1.3 ng g^{-1} dry weight, respectively (Kosubová *et al*., 2005) indicating that the HCH compounds in this region do not exhibit the variations in concentration shown by the pesticide DDT. ΣHCH includes α-, β-, γ- and δ-isomers.

3.1.5 OCP distribution in the investigated matrices

The different values for the HCH family obtained in the investigated matrices are summarized in Table 3-2. α,β-HCH isomers are more stable in soil than γ-HCH. In spite of having lower air concentrations than γ-HCH, the relative amount of α,β-isomers in mineral soil increased, indicating higher persistency in particular for the β-isomer. ε-HCH not detected in the air, was accumulated in the environment in needles as well as in soil. Table 3-2, also shown the efficient OCP accumulation achieved in SPMD, compared to the other studied matrices.

Table 3-2: Concentration values for HCH isomers in the different matrices analysed. Results are the mean values at 4 sampling sites (n=4). The air concentrations are the mean annual values at Haidel. n.d. = non detectable.

Isomer / *Matrix*	*α-HCH*	*β-HCH*	*γ-HCH*	*δ-HCH*	*ε-HCH*
Average air concentration (pg m^{-3})	12.4	0.24	25.0	0.16	n.d.
SPMD ½ year (pg $SPMD^{-1}$)	1882	276.8	5813	89.8	n.d.
SPMD 1 ½ year (pg $SPMD^{-1}$)	2532	363.2	8801	159.7	n.d.
CSB ½ year (pg CSB^{-1})	101.9	1.6	490.7	2.2	n.d.
CSB 1 ½ year (pg CSB^{-1})	92.1	1.8	530.3	6.4	n.d.
Needles ½ year (pg g^{-1} dry weight)	52.1	7.2	180.0	5.4	3.0
Humus soil (pg g^{-1} dry weight)	1107	1816	4458	137.3	39.6
Mineral soil (pg g^{-1} dry weight)	183.3	187.3	228.2	20.1	4.2

The β- and δ- isomers exhibited a better uptake in needles in comparison to the α- and γ-isomers. This suggests a selective uptake in function of the number of axial chlorines presented in the molecule where less axial chlorines (δ-HCH) or the lack of them (β-HCH) is translated in a preferential accumulation in the leaf tissue (Calvelo Pereira *et al*., 2006). In addition, we observed a relatively higher accumulation of β-HCH in the humus layer in comparison to the other isomers. β- and δ-HCH are not so easily desorbed from organic

matter as α- and γ-HCH (Quintero *et al*., 2005). This can cause less availability in this layer to undergo degradation. The isomer ratios changed significantly depending on the matrix analysed, but some similarities were found between the passive samplers, needles and SPMD (Table 3-3). In both of them diffusion processes are involved.

The β-HCH is more easily accumulated in SPMD than the other isomers. This is caused by its K_{oa}, the highest among isomers. In contrast, CSB display more affinity for the γ-HCH than for the β-HCH such as determined by the γ-HCH/β-HCH ratio in table 3-3. It is possible that higher air concentrations, implying a higher concentration gradient for the mass transfer in coated bars, influence positively in the uptake. This assumption could be valid if the coated bar is still acting as an infinite sink.

Table 3-3: Isomer ratios related to the γ-HCH isomer at the different matrices.

Ratio related to γ-HCH / *Matrix*	*γ-HCH/ α-HCH*	*γ-HCH/ β-HCH*	*γ-HCH/ δ-HCH*	*γ-HCH/ ε-HCH*
Air	2.0	104.2	156.3	-
SPMD ½ year	3.1	21.0	64.7	-
SPMD 1 ½ year	3.5	24.2	55.1	-
CSB ½ year	4.8	300	226	-
CSB 1 ½ year	5.8	290	82	-
Needles ½ year	3.5	25.0	33.4	60.2
Humus soil	4.0	2.5	32.5	112.6
Mineral soil	1.2	1.2	11.4	54.8

HCB is almost exclusively found in the gas phase. Its particle association is estimated to be less than 30 % at -10 °C (Shoeib and Harner, 2001). The mean annual value at the Bavarian Forest 67.4 pg m^{-3} is concordant with average air concentrations for the north hemisphere (89 pg m^{-3}). Thus, these values are related to global spreading values for HCB in the Northern hemisphere. HCB is very stable, not only in air but also in soil, due to its completely chlorine substituted aromatic ring. Its hydrophobic properties make it suitable to be accumulated in SPMD as shown in Table 3-4. PeCB with lower K_{oa} than HCB (7.18 and 8.04, respectively) is sequestered in a lower relative amount compared to HCB in SPMD.

Pentachloroanisole is a stable metabolite of HCB, PeCB and pentachlorophenol. As a consequence, it is present in all matrices, in spite of not being used as a pesticide or chemically any more. Octachlorostyrene, a by-product in industry, it is also wide-spread in the environment and detected in all matrices except for needles. SPMD and CSB present

different behaviours regarding these compounds as it was already observed when analysing the HCH family.

Table 3-4: Concentration values for PeCB, HCB, PCAS and OCS in the different matrices analysed. Results are the mean values of the 4 sampling sites (n=4). The air concentrations are the mean annual values at Haidel. n.d. = non detectable.

Compound / *Matrix*	*PeCB*	*HCB*	*PCAS*	*OCS*
Mean air concentration (pg m^{-3})	32.8	67.4	9.9	0.89
SPMD ½ year (pg $SPMD^{-1}$)	524.9	9768	3718	382.8
CSB ½ year (pg CSB^{-1})	51.2	326.1	60.1	10.9
Needles ½ year (pg g^{-1} dry weight)	0.5	85.9	19.0	n.d.
Humus soil (pg g^{-1} dry weight)	723.0	3058	39.6	93.6
Mineral soil (pg g^{-1} dry weight)	297.1	1106	5.5	26.1

The distribution of the chlordane family changed depending on the studied matrix. The metabolite oxy-chlordane was already detected in air at concentrations comparable to the compounds of the technical mixture (Table 3-5). HC and trans-HCE, which are environmentally unstable, were detected in lower amounts or even not at all. Cis-CHL was accumulated at higher concentrations in SPMD than trans-CHL. This could be related to its spatial distribution that favours the transport through the membrane.

Table 3-5: Concentration values for trans-CHL, cis-CHL, oxy-CHL, HC, trans-HCE and cis-HCE in the different matrices analysed (n=4). The air concentrations are the mean annual value at Haidel. n.d. = non detectable.

Compound / *Matrix*	*trans-CHL*	*cis-CHL*	*oxy-CHL*	*HC*	*trans-HCE*	*cis-HCE*
Mean air concentration (pg m^{-3})	0.40	0.66	0.50	0.11	0.02	1.68
SPMD ½ year (pg $SPMD^{-1}$)	179	535	302	2.9	n.d.	852
CSB ½ year (pg CSB^{-1})	6.4	22.9	7.7	n.d.	n.d.	43.0
Needles ½ year (pg g^{-1} dry weight)	2.0	4.0	2.3	n.d.	n.d.	12.6
Humus soil (pg g^{-1} dry weight)	438	377	264	6.1	11.3	1345
Mineral soil (pg g^{-1} dry weight)	63.4	45.2	52.9	2.0	2.5	219

Dieldrin concentrations in the environment are not only a consequence of direct historical use as a pesticide but also to the use of aldrin because dieldrin is a breakdown product of it. Dieldrin residues in the mineral soil result from both pesticides and are in higher amounts than the aldrin residues (Table 3-6). The air concentrations of endosulfan were α- dominant, not only because the technical mixture has a major quantity of it, but also because the β- is transformed slowly to the α-isomer at aqueous-air interfaces (Schmidt *et al*., 2001). In

needles, the opposite tendency was observed. This could be related to the micro-organism activity on leave surfaces, where the β-isomer is more resistant to oxidation than the α-isomer (Sutherland *et al*., 2004). In soils, the amounts of α-endosulfan were significantly lower than the β-endosulfan. This is also related to the specific isomer characteristics: α- is an order of magnitude more volatile than the β-endosulfan. Thus, accumulation of the less volatile isomer can be observed in the humus soil as well as in the mineral soil. The β-endosulfan amount increased significantly in relationship to the α-endosulfan from the air to soil compartments ranging through SPMD and needles as indicated by the α/β isomer ratio. The pesticide mirex exhibited low air concentrations and is accumulated in the soil compartment due to the low vapour pressure and low water solubility. This perchlorinated compound, thus very stable and with its high lipophilicity, has a very high uptake in SPMD (Figure 3-6). On the other hand, methoxychlor, detectable neither in air nor in SPMD, presents an accumulation in soils of the same order as mirex. Methoxychlor is characterised by a very low vapour pressure and by binding tightly to the soil, as indicated in the different compound distributions obtained for the matrices studied. CSB deployed 1/2 year are not able to detect chlorine pesticides exhibited at very low air concentrations (lower than 0.05 pg m^{-3}).

Table 3-6: Concentrations for some pesticides in the different matrices analysed. The results are the mean values of the 4 sampling sites. The air concentrations are the mean annual value at Haidel. n.d. = non detectable.

Compound / *Matrix*	*Aldrin*	*Dieldrin*	*α-endosulfan*	*β-endosulfan*	*Ratio α/ β*	*Methoxychlor*	*Mirex*
Mean air concentration (pg m^{-3})	0.02	2.33	28.4	2.35	12	n.d.	0.05
SPMD ½ year (pg $SPMD^{-1}$)	3.4	1830	61891	14408	4.3	n.d.	49.3
CSB ½ year (pg CSB^{-1})	n.d.	62.8	1542	135.4	11	n.d.	n.d.
Needles ½ year (pg g^{-1} dry weight)	0.3	20.7	69.6	77.5	0.9	4.1	n.d.
Humus soil (ng g^{-1} dry weight)	24.6	789.6	6.8	13.2	0.52	8.4	13.4
Mineral soil (ng g^{-1} dry weight)	5.7	3821	121.6	490.1	0.25	94.0	84.3

DDT family

From the DDT family, p,p'-DDT and p,p'-DDE compounds were predominant in the environment. In particular, p,p'-DDT and its most stable metabolite p,p'-DDE were the main compounds in all matrices (Table 3-7). In the atmosphere p,p'-DDT is dehydrohalogenated relatively fast to produce p,p'-DDE (Rahm *et al*., 2005). Therefore, it was the predominant metabolite detected in all the seasonal samplings.

Table 3-7: Concentration of DDT and its derivatives in the different matrices analysed (n=4). The air concentrations are the mean annual values at Haidel. n.d. = non detectable.

Compound / *Matrix*	*p, p′-DDT*	*o, p′-DDT*	*p, p′-DDD*	*o, p′-DDD*	*p, p′-DDE*	*o, p′-DDE*
Mean air concentration (pg m^{-3})	1.05	1.09	0.22	0.20	6.3	0.38
SPMD ½ year (pg $SPMD^{-1}$)	460	354	129	66	2208	47
CSB ½ year (pg CSB^{-1})	56.4	14.0	n.d.	n.d.	129.3	4.4
Needles ½ year (pg g^{-1} dry weight)	87.7	21.9	2.0	0.9	59.5	0.7
Humus soil (pg g^{-1} dry weight)	23543	4662	119	n.d.	10070	118
Mineral soil (pg g^{-1} dry weight)	6441	1509	39.5	12.2	4856	39.4

The semi permeable device showed to be the matrix with most similarities to the air pool. This can be seen in Table 3-8 by comparing the calculated ratios. Very similar ratios were also found in semi-rural Canadian areas using passive samplers. The ratios p,p'-DDT/p,p'-DDE and p,p'-DDT/o,p'-DDT were 0.43 and 1.25, respectively (Harner *et al*., 2004). Both ratios, in the same range, were obtained in air (0.2 and 1.0) and SPMD (0.4 and 1.4) in this study. In needles, the pattern is different. This could be related to the waxy reaction matrix at the needle surface where photolysis of DDT occurs (Dolinováa *et al*., 2004). In humus, a high amount of parent compounds is observed. This can be explained by the soil characteristics. The higher amount of organic matter causes more absorption of the pesticide into it which is thereby sequestered and not available for soil degradation processes. This effect increases in the humus soil, a fact that has also been observed in another forest sampling site close to the actual site under investigation (Kosubová *et al*., 2005).

Table 3-8: Amount of DDT compounds related to o, p'-DDT in the matrices analysed. The results are the ratios of the concentrations.

Ratio o,p′-DDT/compound / *Matrix*	*o, p′-DDT/ p, p′-DDT*	*o, p′-DDT/ p, p′-DDD*	*o, p′-DDT/ o, p′-DDD*	*o, p′-DDT/ p, p′-DDE*	*o, p′-DDT/ o, p′-DDE*
Mean air concentration	1.0	4.9	5.3	0.20	2.7
SPMD ½ year	0.71	5.6	8.6	0.29	8.6
CSB ½ year	0.25	-	-	0.56	3.3
Needles ½ year	0.25	10.8	23.5	0.38	33.8
Humus soil	0.20	39.4	-	0.46	39.8
Mineral soil	0.23	37.9	122	0.30	37.9

3.2 PAH in the Bavarian and Bohemian forests

The section 3.2.1 is based on unpublished data and section 3.2.2 in paper I.

3.2.1 PAH distribution in the investigated matrices

The percent values obtained in the studied matrices for each compound are summarized in Table 3-9. Higher percentage of high molecular weight PAH was accumulated in soil than in the other matrices. High molecular weight PAH presented higher values of organic-carbon partition coefficient than low molecular weight PAH (Wong *et al*., 2004). Elevated organic-carbon partition coefficients cause more retention of the compound to the soil. This effect was observed in both soils analysed: mineral and humus. The increasing vapour pressure at lower molecular weight PAH is also a property that determines the observed pattern.

Table 3-9: Percentage values for selected PAH in the different matrices analysed. Results are given considering the mean of the 4 sampling sites excepting the air concentrations (only at Haidel). n.d. = not detectable.

Matrix / *Compound*	*Air*	*SPMD ½*	*SPMD 1 ½*	*Needles ½*	*CSB ½*	*Mineral*	*Humus*
AC	2.1	0.87	0.27	0.60	1.0	0.31	0.30
ACL	2.7	n.d.	n.d.	0.35	0.23	0.07	0.14
FL	19.6	7.6	5.4	2.3	15.7	0.13	0.21
PHE	41.0	50.1	37.7	11.3	45.8	2.9	4.3
AN	0.4	n.d.	n.d.	0.36	n.d.	0.35	0.39
FA	14.1	27.0	44.7	23.0	31.7	7.6	11.8
PY	5.7	6.9	6.7	12.6	3.2	4.8	6.8
BbnT	0.1	0.52	0.16	0.75	0.08	0.58	0.81
BaA	1.2	0.44	0.38	3.4	0.10	2.0	2.4
Chr	2.0	1.46	1.75	8.0	0.88	13.7	10.6
BbFA	3.9	1.62	0.55	11.3	0.52	30.9	29.6
BkFA	1.3	1.20	0.99	4.1	0.23	6.8	5.5
BeP	1.7	0.78	0.53	6.0	0.16	9.6	9.1
BaP	1.4	0.35	0.26	4.6	0.05	2.0	2.9
PE	0.2	0.06	0.05	0.75	0.02	0.44	0.58
IP	1.3	0.70	0.34	5.8	0.16	9.3	7.3
DBahA	0.3	0.03	0.03	1.1	0.09	3.5	2.3
BghiP	1.1	0.31	0.19	3.7	0.15	4.9	4.9

Compounds with higher vapour pressure tend to remain in the air (gaseous phase) and not in the leaf or soil compartment. Additionally, high molecular weight PAH are more resistent to biodegradation than lower molecular weight PAH in the soil and water compartment (Cerniglia, 1992).

The distribution of PAH at the 4 sampling sites is depicted (coded as GSF) and set in comparison with soil analytical determinations performed at the closely related geographical sites by the Bavarian Environmental Agency (Landesamt für Umwelt: LfU) as shown in Figure 3-7. The values are comparable either in total amounts or in compound distribution for all the sampling sites except for the BbFA and BkFA compounds (depicted in yellow and in sky blue, respectively). This difference could be related to the lack of chromatographic resolution between these two compounds during analysis at LfU.

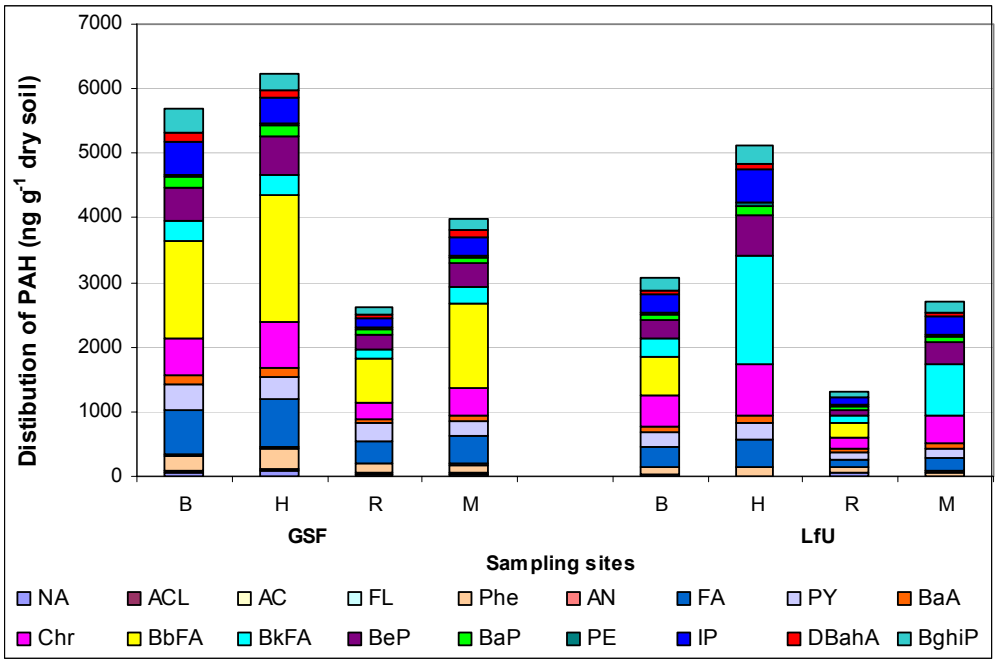


Figure 3-7: Concentration of PAH in humus soil at four spatially closely related sites. Analysis carried out at two independent laboratories. H: Haidel, B: Boubin, R: Ruckowitzschachten, M: Mitterfels.

It has to be kept in mind that the compounds analysed in needles correspond to the amounts extracted from the leaf cuticle as well as the amounts accumulated by dry and wet deposition on the plant surface. Compounds with higher vapour pressure and lower molecular weight are able to penetrate more into the leaf (Umlauf *et al*., 1994, Kaupp *et al*., 2000). Percentage values of high molecular weight PAH in needles are in between the air and soil percentage values. One exception is BaP and BaA with higher percentage amounts in the leaf compartment than in the other matrices, including mineral and humus soil. Although, BaP can

be degraded by photolysis and oxidation, biodegradation is the main degradation process for this compound (Dzul-Puc *et al*., 2005). This could be one reason why this compound exhibited lower amounts in the soil compartment in relation to other high molecular weight PAH at similar air concentrations.

In the case of PHE, FA and PY the amounts in the leaf compartment were also higher than in the soil compartment. PHE percentage decreased abruptly in the soil in comparison to the air percentage. Biodegradation studies with PHE, FA and PY showed a faster biodegradation for PHE than FA and PY (Pagnout *et al*., 2006). This is congruent with the relative lower amount of PHE detected in soil than for FA and PY.

Analysing the percentage distribution of PAH in SPMD reveals a predominance of low molecular weight PAH that were also predominant in air. The sampling based on diffusion processes through the membrane determines this characteristic pattern. The size selection favours the mass transfer of low molecular weight PAH through the membrane. As a consequence, compounds with lower molecular volume and mainly associated to the vapour phase as PHE, PY and FA were well detected with this device. It is worth to mention the good uptake that FA presented in the SPMD device which proves to be an ideal device to detect this compound (K_{oa}=8.76). Under these conditions also higher concentrations of FL in the SPMD device would be expected but such an accumulation effect was not observed (K_{oa}=6.90). On the other hand, FL showed a very efficient uptake in CSB where sorption mechanisms are predominant. Fluorene biodegradation occurs quickly under oxygenated conditions and this determined its readily biodegradation in soil and needles. Its biodegradation probably also influenced the lower accumulation obtained in SPMD where FL uptake is not as fast as FL degradation. Additionally, compounds with higher K_{oa} and lower Henry's Law constant were well collected not only in SPMD but also in needles. In needles, a separation related to the molecular weight of PAH as in SPMD was not observed. It is remarkable, that high molecular weight compounds, present only in the particulate phase, were found in the needle samples.

To exemplify and reinforce this observation, PAH results from the Bavarian Forest carried out independently at the German Research Center for Environmental Health (coded as GSF) and at the Federal Environmental Agency of Germany (coded as UBA) are shown below. Both sampling sites are located close to each other and to the mountainous barrier (Kirchner *et al*. 2006, Rappolder *et al*. 2007). Spruce needles from the sampling site Haidel (1160 m a. s. l.) and the Federal Environmental Agency site R5 (1240 m a. s. l.) were collected consecutively for 3 years. In spite of the good uptake of high molecular weight PAH, the compounds, PHE, FA and PY predominated in the samples independently of the needle age (Figures 3-8 and

3-9). This compound distribution was also observed by Schröter-Kermani *et al*. (2006) at urban and rural areas in Germany.

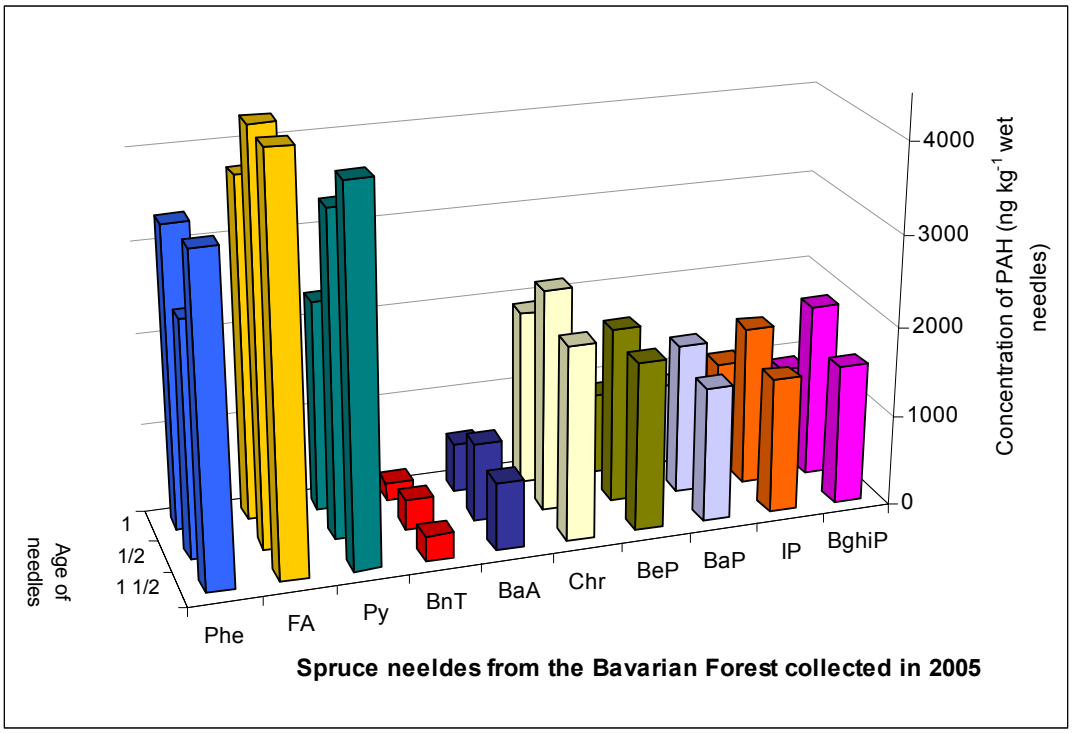


Figure 3-8: Selected PAH quantified in 1 year old needles at R5 and ½ and 1 ½ years old needles at Haidel. Needles collected in 2005.

This similarity is detected not only spatially but also temporally. The samplings in the years 2004 and 2005 presented the same trend as shown below for the year 2003 (Figure 3-9).

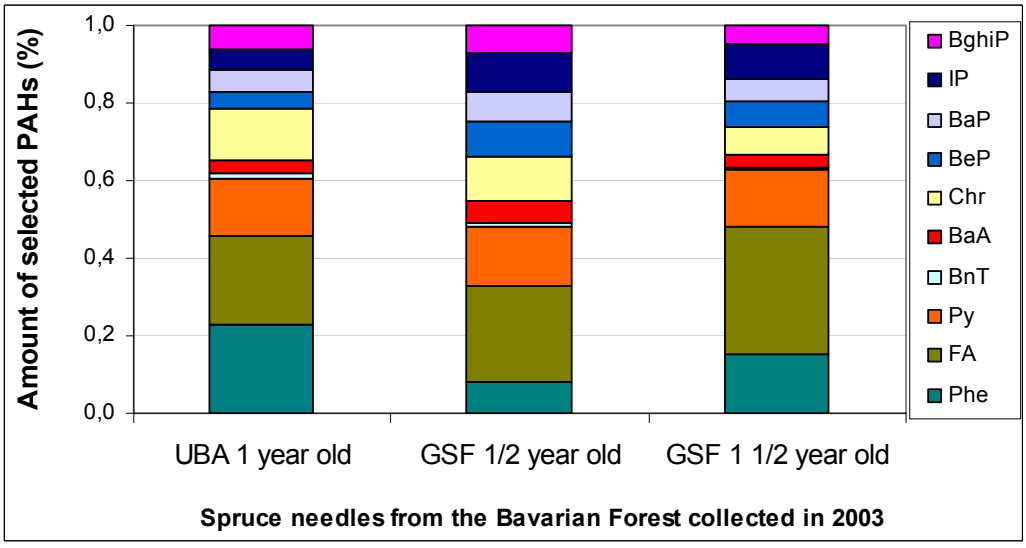


Figure 3-9: Percentage distribution of selected PAH in needles collected at different ages at the Bavarian Forest. UBA 1 year old: needles of 1 year of age at R5. GSF: ½ and 1 ½ years old needles at Haidel.

3.2.2 Comparison of PAH concentrations in SPMD, active sampler, and spruce needles

PAH: Needles and active sampler

Regarding the active samplers, the whole PAH family showed the same trend to a concentration increase in early and late winter and a decrease of concentration in the warmer seasons. This is for instance, particularly remarkable for the heavy molecular weight PAH, e.g.: BbFA, BkFA, BeP and BaP presenting a strong correlation between them with at least r^2=0.93. The low molecular weight PAH correlated, neither among them nor with the heavier molecular weight PAH. Higher concentrations of PAH are determined during the coldest periods (blue circle), whereas the lower concentrations are detected in summer (red circle) as shown in Figure 3-10.

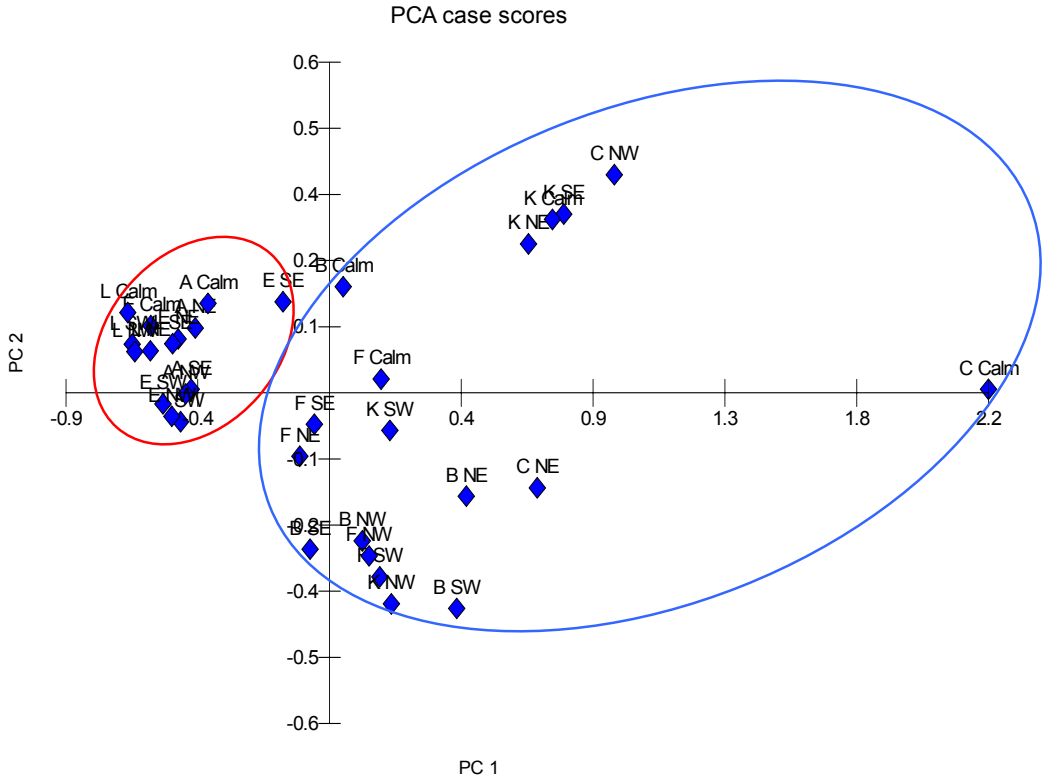


Figure 3-10: PCA case scores (33) of PAH (16) for the active sampling campaigns A, B, C, E, F, K and L. PC 1 accounts for 80.4 % and PC 2 for 8.4 % of the variability (88.8 % of the total).

To illustrate this with an example, PHE reached the highest air concentrations in late winter with 4.7 ng m^{-3} in comparison to 0.8 ng m^{-3} observed in summer, meanwhile the carcinogenic BaP fluctuated from 0.12 to 0.014 ng m^{-3} in air. In addition, we notice a low variation of PAH concentrations in the warmer periods where all the wind directions for campaigns are grouped

inside the red circle. This cluster indicates PAH background values in the air concentrations and no specific advective input during the warmer seasons. However, winter campaigns showed higher PAH air concentrations as well as higher loading fluctuations in the different wind directions than in summer. We can conclude that the air concentrations in winter are influenced by local and regional input sources related to winter activities such as domestic heating not present during the warmer periods.

Previous studies in a semi urban zone showed as well such a temporal trend (Kaupp and McLachlan, 1999). FL and PHE are the compounds detected in higher concentrations in all sampling campaigns. PHE represents in the studied campaigns between 33-50 % of the whole PAH. The compounds FA and PY are also present in all the sampling campaigns with percentages between 9.5-14.5 % and 4.5- 6.8 %, respectively. The low molecular weight PAH are in higher concentrations than high molecular weight PAH.

In needles, no general trend is observed, but some correlations are found between compounds with similar physicochemical properties. We observe for instance, a correlation between Pyrene (PY) and Fluoranthene (FA) in needles with r^2=0.93. These compounds have similar properties such as surface area, molecular volume, water solubility and $K_{oa.}$ The prediction of vegetation-atmosphere partition coefficients, gave very similar values for them (Simonich and Hites, 1994, Maddalena *et al*., 2002). Consequently, this can explain the similar behaviour found in needles for these compounds. Perylene (PE) correlates with BaP (r^2= 0.95), which was to be expected due to their physicochemical similarities as well as to their similar vegetation-atmosphere partition coefficients (Alves de Lima Ribeiro *et al*., 2003). PAH relative distribution in needles is different to the one determined in air (Figure 3-11).

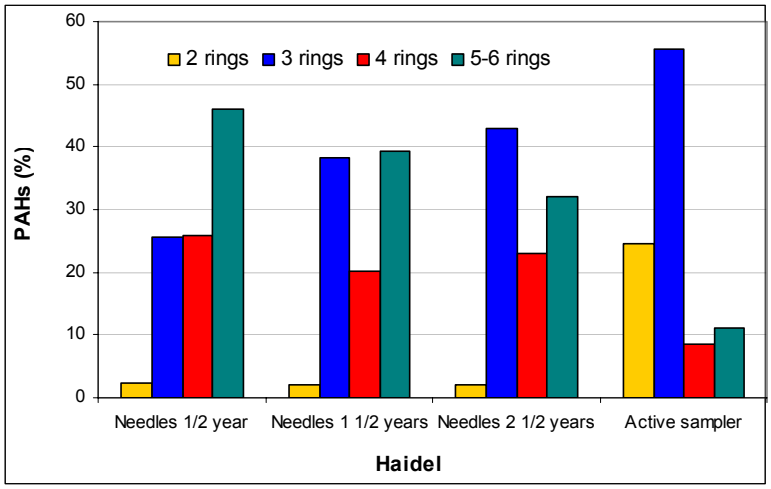


Figure 3-11: PAH distribution (%) in needles ½, 1 ½ and 2 ½ years old and active sampler at Haidel. Sampling October 2003. Classification according to the number of benzene rings (BbFA, BkFA included in 5-6 rings).

Low molecular weight PAH, such as PHE, FL and ACL are present in air in relatively higher concentrations than in needles. However, intermediate and high molecular weight PAH tend to accumulate in higher percentages in needles than low molecular weight PAH. The predominant compound in needles is FA, representing more than 20 % of the whole PAH concentration, while PHE, one of the predominant compounds in air, decreases in the leaf compartment to a value around 12 % of the whole PAH. This percentage distribution is observed at the four sampling sites during the periods October 2003 and October 2004. If we compare the PAH concentration distribution in needles and air at Haidel, we see that higher amounts of 5 and 6 ring PAH are found in needles of all ages than in air. Furthermore, the 2 benzene ring compounds are in lower percentage in needles than in air. Thus, less accumulation of low molecular weight PAH is observed in the leaf compartment compared to the high molecular weight PAH. There is no correlation between concentrations of PAH in air and in needles.

Performing PCA, we observe a tendency to higher accumulations at older needle ages but also high fluctuations among sampling sites and the year of collection (Figure 3-12).

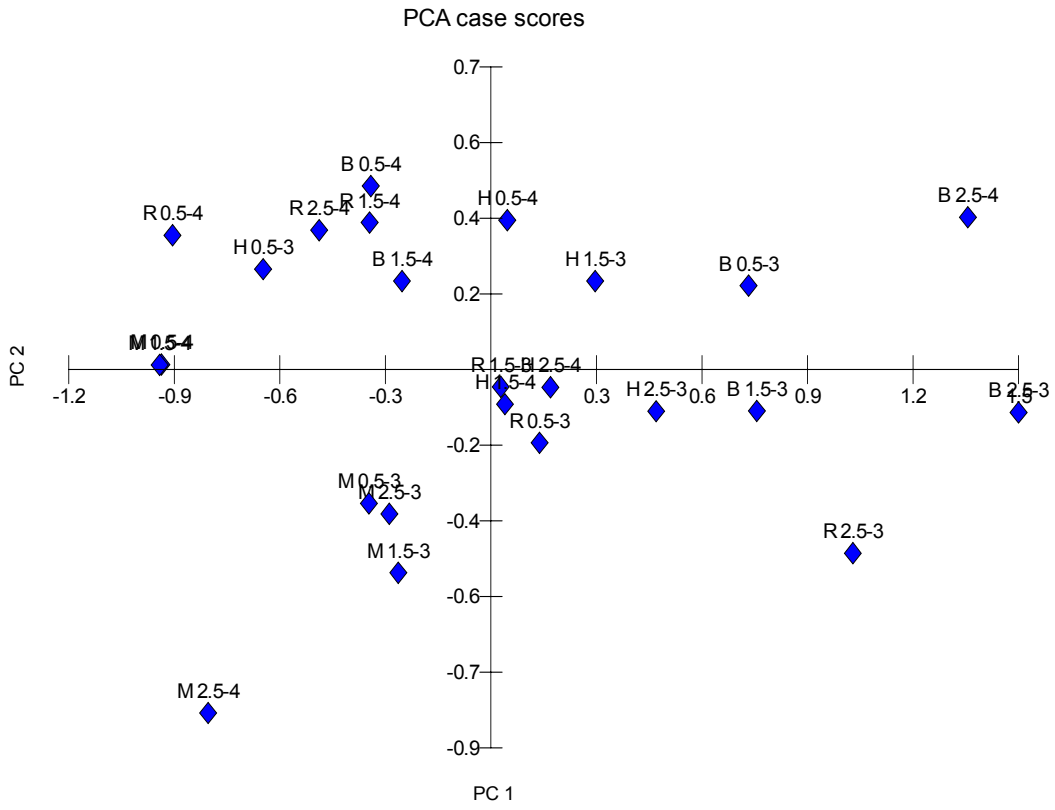


Figure 3-12: PCA case scores (24) of PAH (18) for needles. 2.5: 2 ½ years old, 1.5: 1 ½ years old and 0.5: ½ year old. 3: year 2003 and 4: year 2004. PC 1 accounts for 59.3 % and PC 2 for 13.8 % of the variability (73.1 % of the total).

PAH: SPMD and needles as abiotic and biotic samplers

In SPMD, two groups presented correlations: Chrysene and Fluoranthene on the one hand and BaA, BeP, BaP, PE and BghiP on the other hand. The correlation in both groups can be explained by similar chemical physical properties: Chr and FA are characterized by comparable hardness values (Alves de Lima Ribeiro *et al*., 2003) implying similar chemical stability. In addition, properties such as surface area, volume and K_{oa} are similar among the compounds in both groups. This suggests that properties like spatial molecular distribution, stability of the compound and hydrophobicity, influence the PAH uptake process in the SPMD for these compounds.

When analysing the distribution of PAH related to their number of aromatic rings in SPMD, no significant differences for ½ year and 1 ½ years exposure were found (Figure 3-13). Moreover, three aromatic rings PAH exhibited in major proportions in air, result to be also dominant in SPMD devices (Figure 3-13).

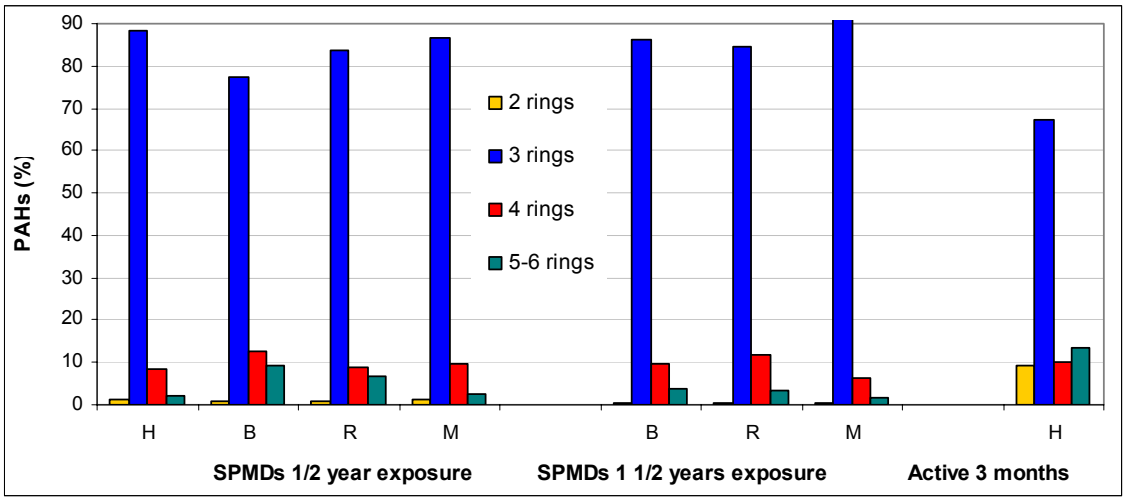


Figure 3-13: PAH distribution (%) in SPMD for ½ year and 1 ½ years exposure at 4 sampling sites and active sampler at Haidel. Site codes: H: Haidel, B: Boubin, R: Ruckowitzschachten and M: Mitterfels.

Comparing SPMD with needles we see that more low molecular weight PAH are accumulated in SPMD than in needles in the same time period. Actually, the heavy molecular weight PAH are mainly found in the particulate phase, being this characteristic an impediment for the uptake of these compounds through the membrane device. This explains why more percentage of 3 aromatic ringed PAH related to the whole sample are found in SPMD than in needles. In spite of this fact, the compound Benzo(b)naphtho(2,1-d)thiophene (3 aromatic ringed PAH) is found in needles and SPMD 1 ½ years exposed but not in SPMD of shorter exposition time. This compound is characterized by being the only studied compound containing sulphur. The

electrophilicity of this element creates a negative charge and as a consequence higher molecular polarity than in the other studied PAH. As a consequence, the uptake through the SPMD membrane can be hindered.

The 3 aromatic ringed PAH group is composed of Phenanthrene and Fluoranthene (Anthracene is not considered due to lack of chromatographic resolution and their small quantities) where PHE represents in average almost 50 % of the whole PAH presented in the samples and FA around 25 % in SPMD exposed for ½ year. Bartkow *et al.* (2004) quantified PHE as the main compound of PAH (around 40 %) in SPMD deployed for 32 days. In SPMD exposed 1 ½ years the increased proportional amount of 3 ringed PAH is due to an increase of FA that reached more than 30 % of the total PAH in the sample.

Low molecular PAH presented a correlation between active and SPMD sampling. Even high molecular weight PAH, bound to particular material, correlated in both, SPMD and active samplers. Nonetheless, due to the low concentration of high molecular weight PAH, more studies for these compounds are necessary for SPMD (Figure 3-14).

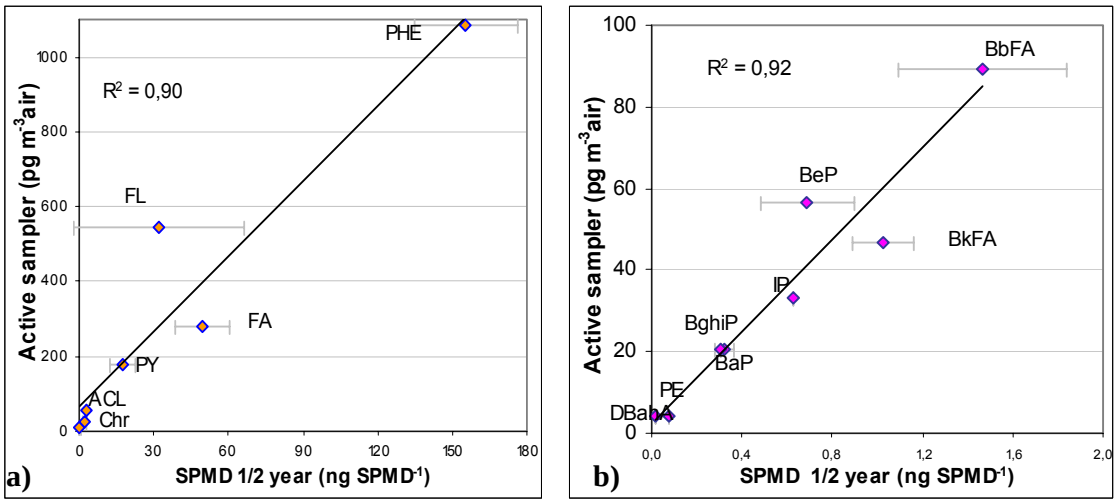


Figure 3-14: PAH concentrations in SPMD ½ year exposure (n=2) and active sampler 3 month exposure at Haidel. a) Low molecular weight PAH b) High molecular weight PAH.

3.3 PCDD/F in the Bavarian and Bohemian forests

This section is based on Paper II.

3.3.1 PCDD/F: Needles and Active sampler

The homologue distribution for dioxins and furans in spruce needles is shown in Figure 3-15. The distribution at the four sites showed a PCDD percentage increase towards the higher chlorinated congeners in needles.

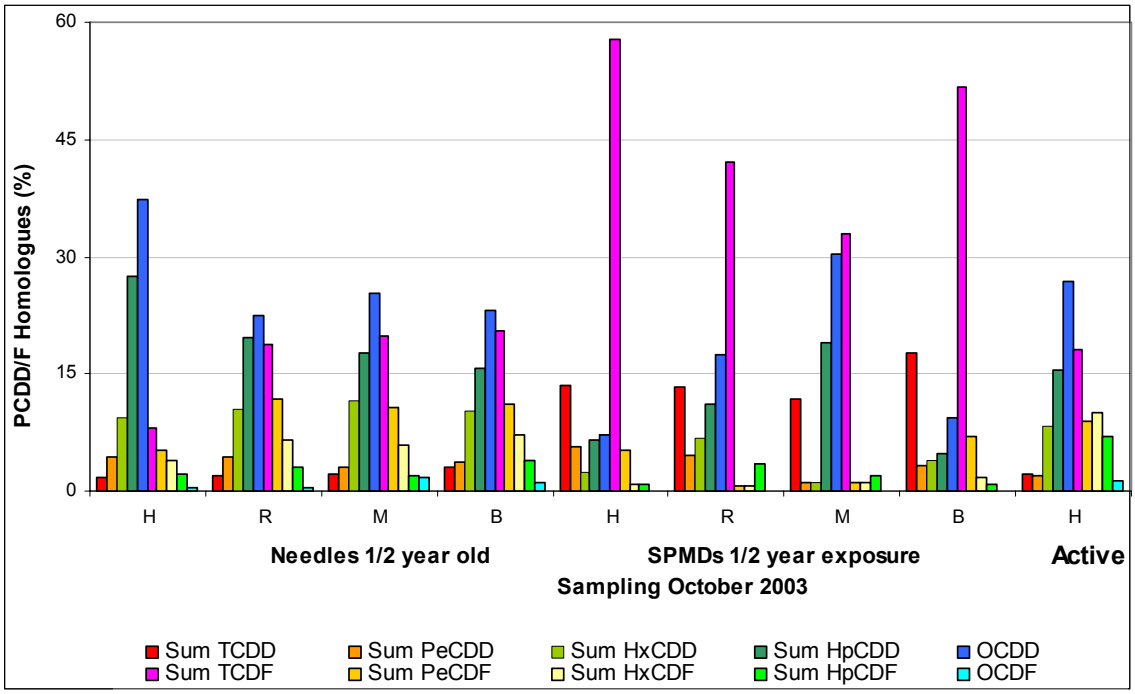


Figure 3-15: PCDD/F homologues distribution (%) in ½ year old spruce needles and SPMD exposed for ½ year at H: Haidel, R: Ruckowitzschachten, M: Mitterfels and B: Boubin and in active sampling, campaign A at Haidel. Sampling October 2003.

The trend for PCDF was the opposite one; lower percentage was found for higher chlorinated congeners. This pattern was observed in all needles where less percentage differences between OCDD and ΣTetraCDF were found in aged needles (Figure 3-16). Therefore, the homologue distribution of PCDD/F is characteristic in all the collected needles with an increase of ΣTetraCDF related to OCDD in aged needles. Comparing the four sampling sites, a higher percentage of ΣHeptaCDD and OCDD was detected at Haidel. Atmospheric bulk deposition studied by Horstmann *et al*. (1997) in a spruce forest near Bayreuth (North-Eastern Bavaria) exhibited the same homologue pattern where the maximal concentration values were also determined for OCDD. Active sampling in Southern Bavaria close to an urban area (Augsburg) performed by Hippelein *et al*. (1996) reported on average a similar homologue profile.

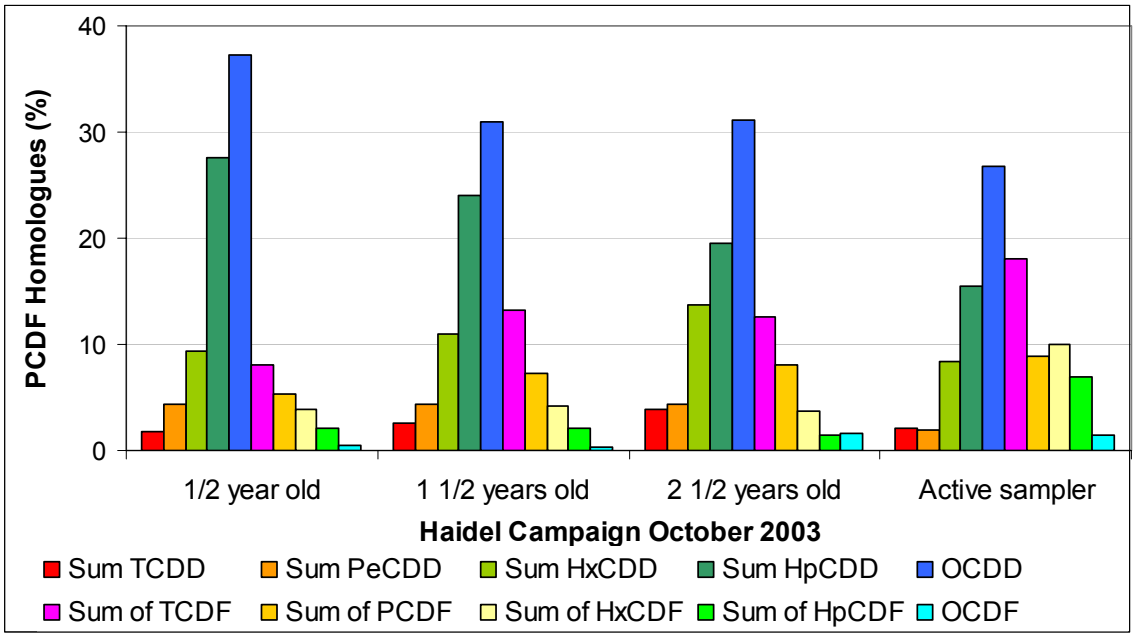


Figure 3-16: PCDD/F homologues distribution (%) in ½, 1 ½ and 2 ½ years old spruce needles and active sampling, campaign A at Haidel. Sampling October 2003.

PCDD/F homologue profiles determined in spruce needles by Rappolder *et al*. (2007) at locations (Eastern Bavaria and Sumava) close to the monitored sites in the present study showed a similar PCDD/F trend where the main percentage of homologue compounds is shifted from OCDD to ΣTetraCDF. PCDD air concentrations increased from lower to higher chlorinated homologues. Nonetheless PCDF air concentrations tend to decrease at higher chlorine substitutions (Figure 3-16). Concentrations of PCDD homologues measured in spruce needles and active sampling trials taken from the same location showed a strong correlation (Figure 3-17a). A similar correlation was also achieved for older spruce needles and the active sampling (r^2 =0.96 and r^2=0.98 for 1 ½ year and 2 ½ years old needles, respectively) pointing out the similar pattern of PCDD found in both sampling methods. PCDD congeners (Figure 3-18a) mark the same pattern found in PCDD homologues (Figure 3-17a). The congeners in this graphic were chosen considering the compounds that were always detectable or presented low numbers of non detectable values in the analysed samples. Needles with older ages tend to accumulate compounds (Piccardo *et al*., 2005), (Herceg and Krauthacker, 2006) showing seasonal differences during the year (Kylin and Sjödin, 2003). In this study, the bioaccumulation of PCDD/F is observable in older needles in both samplings of October 2003 and 2004. The needles were collected in the earlier autumn season after the summer period where compounds partially bound to particles tend to show a higher accumulation than in the rest of the annual seasons (Hellström *et al*., 2004). The fact that

higher chlorinated PCDD are at higher air concentrations than lower chlorinated PCDD determines the pattern obtained in needles but the season of the sampling also influences the obtained results for PCDD. For PCDF at older needle ages the correlation between air and needle concentrations decreased clearly (Figure 3-17b) due to the different shift of PCDF homologues towards higher concentrations. Hence, we can not infer a direct relationship between the active sampling and needle concentrations for aged needles.

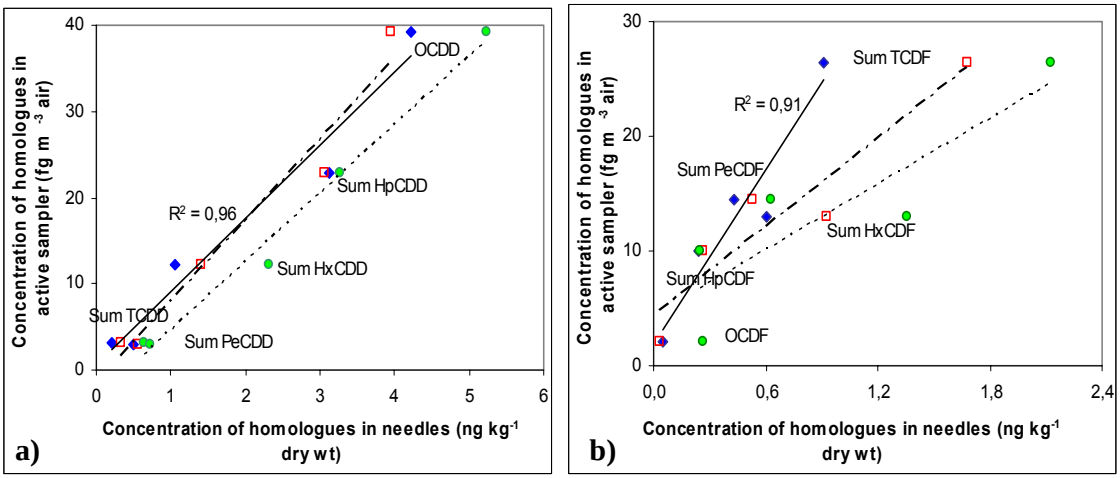


Figure 3-17: a) PCDD and b) PCDF homologue concentrations of spruce needles and active sampler (campaign A, 3 months exposure) at Haidel. Diamonds, squares and circles represent ½, 1 ½ and 2 ½ needle ages, respectively. Sampling October 2003. Correlation coefficient between active sampling and ½ year old needles.

Despite of this, an agreement between both methods for all homologues was found for ½ year old needles. Regarding PCDF congeners, only a correlation between the younger needles and the active sampler could be found. This indicates that the concentration distribution for PCDF in needles is changing with time, whereas this change was not observed for PCDD. This difference obtained for older needles for PCDF could be attributed to the lower chlorinated compounds TetraCDF and PentaCDF can be accumulated more easily into the leaf compartment than higher chlorinated PCDF homologues due to first, higher air concentrations and second, higher availability in the gaseous phase. The fact that during the sampling period the absorption of higher chlorinated compounds is favoured can not counteract the higher air concentrations and the higher availability in the gaseous phase of the TetraCDF and PentaCDF. Another influencing factor is the persistence of the compound. Recent studies indicated a possible increase of lower chlorinated congeners due to photolytic dechlorination (Niu *et al*., 2003) where PCDF tend to be more reactive than the PCDD. Thus, the higher amounts of PentaCDF and TetraCDF may also be related to an increase of photochemical degradation products accumulated or formed in the needle surface at longer sunlight exposure

(Figure 3-18b). Another variable to take into account is the mobilisation of the compounds into the leaf compartments. The work performed by Kaupp *et al*. (2000) suggested that from the PCDD/F accumulated in the cuticle and classified as particle bound fraction, PCDD are more immobile than PCDF in maize leaves. If this could also be applied to the needle compartment it may be another factor that implies the major stability in the pattern obtained for PCDD in comparison to PCDF.

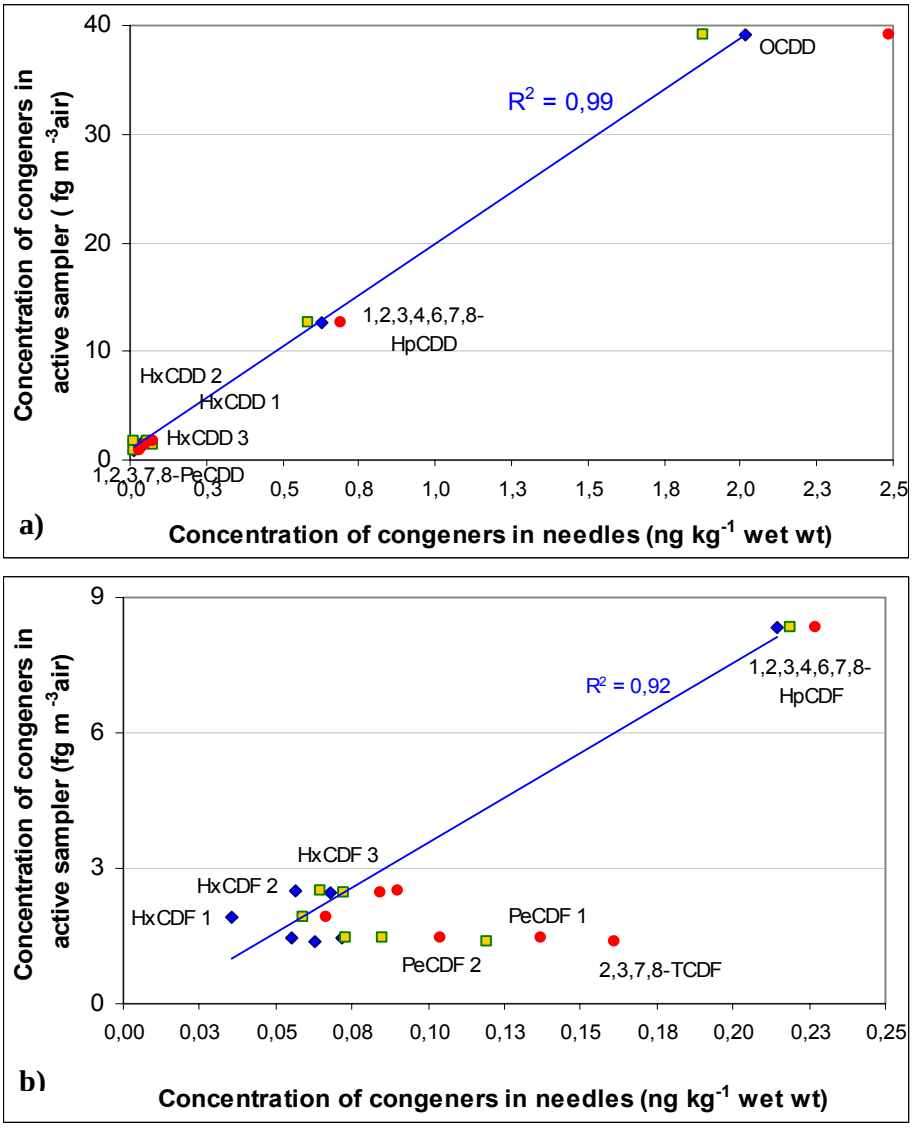


Figure 3-18: a) PCDD congener concentrations in spruce needles and active sampler. HxDD 1: 1,2,3,6,7,8-HxCDD, HxDD 2: 1,2,3,7,8,9-HxCDD, HxDD 3: 1,2,3,4,7,8-HxCDD. b) PCDF congener concentrations in spruce needles and active sampler. PeCDF 1: 1,2,3,7,8/1,2,3,4,8-PeCDF, PeCDF 2: 2,3,4,7,8-PeCDF, HxCDF1: 1,2,3,4,7,8/1,2,3,4,7,9-HxCDF, HxCDF 2: 2,3,4,6,7,8-HxCDF, HxCDF 3: 1,2,3,6,7,8-HxCDF. Sampling October 2003. Correlation coefficient between active sampling (Campaign A, 3 month exposure) and ½ year old needles. Diamonds, squares and circles represent ½, 1 ½ and 2 ½ needle ages, respectively.

3.3.2 PCDD/F: SPMD and Needles as abiotic and biotic passive samplers

The PCDD/F homologue concentrations obtained from SPMD samples were different from those related to spruce needles. The PCDD/F homologue distribution (in % of the total amount of PCDD/F) is shown in Figure 3-13. OCDF was not detectable in the majority of the samples. Taking into account that the SPMD is able to sample mainly the gaseous and the nano-particulate phase but not the contaminants attached to macro-particulate aerosol the different homologue distribution obtained is not surprising. Homologues with comparable air concentrations but different partitioning behaviour had different distributions in these two passive samplers. This is the case for HeptaCDD and TetraCDF homologues where their air concentrations are similar (and the highest after OCDD air concentrations). However, their distribution in both passive samplers is clearly different. HeptaCDD tends to be mainly bound to particles (69 to 100 %) during all seasons meanwhile TCDF is mainly present in the gaseous phase (Hippelein *et al*., 1996). For this reason, HeptaCDD homologues able to achieve similar percentage amounts as the TetraCDF homologues in needles are significantly different in the SPMD pattern. Experimental studies in a semi rural field station showed that 2,3,7,8 TetraCDD/Fs present a higher proportion in the gaseous phase in comparison to the Penta to OCDD/F (Harner *et al*., 2000). Therefore, higher concentrations of the tetra congeners in the gas phase reflect the pronounced uptake of these compounds into SPMD in comparison to the spruce needles. The tetra homologues peaked in the distribution of all sampling sites. An increase for the Penta and HexaCDD/F in comparison to the HeptaCDD/Fs and OCDD is also observable (Figure 3-17). Harner *et al*. (2000) suggested the division of PCDD/Fs in two groups, according to their chlorine substitution pattern where the group 2 (any Tetra to HexaCDD/F with 3 or 4 chlorines in the 2,3,7,8 substitution) is characterised for uniform charge distribution in comparison to the group 1 (the rest of PCDD/F). The lower polarity exhibited in group 2 reflects a higher octanol solubility, and thus a higher K_{oa} for this PCDD/F group. This implies a better uptake into the SPMD device for the group 2 in comparison to HeptaCDD/Fs and OCDD/F that belong to the group 1. On the other hand, SPMD exposed for 1 ½ years do not show linear increase of PCDD/F concentrations compared to the 1/2 year exposed devices but a relative increasing of Tetra to HexaCDF compounds (Figure 3-17). ΣTetraCDD and ΣPentaCDF increase significantly, even the ΣHexaCDF, close to the limit of detection after ½ year exposure is well determined at longer exposure periods. This is in agreement with the study of Schröder *et al*. (1997) where it was determined that of the whole PCDD/Fs homologues dry gaseous deposition occurs for TetraCDD and Tetra to HexaCDFs. A comparison of the homologue patterns of SPMD

(Figure 3-19) and needles (Figure 3-16) underline the importance of this process in the uptake of compounds in SPMD. It has to be considered that longer periods allow the mass transfer from the particles attached to the membrane and the membrane itself into the SPMD. The homologue distribution of SPMD presented a similar pattern after 1 ½ years exposure at all sampling sites, which was less clearly observed for SPMD exposed ½ year (Figure 3-19). Extended exposure periods can cause non-linear uptake. Hence, such prolonged periods are not advisable for sampling of PAH if a kinetic uptake (linear uptake) with SPMD is desirable.

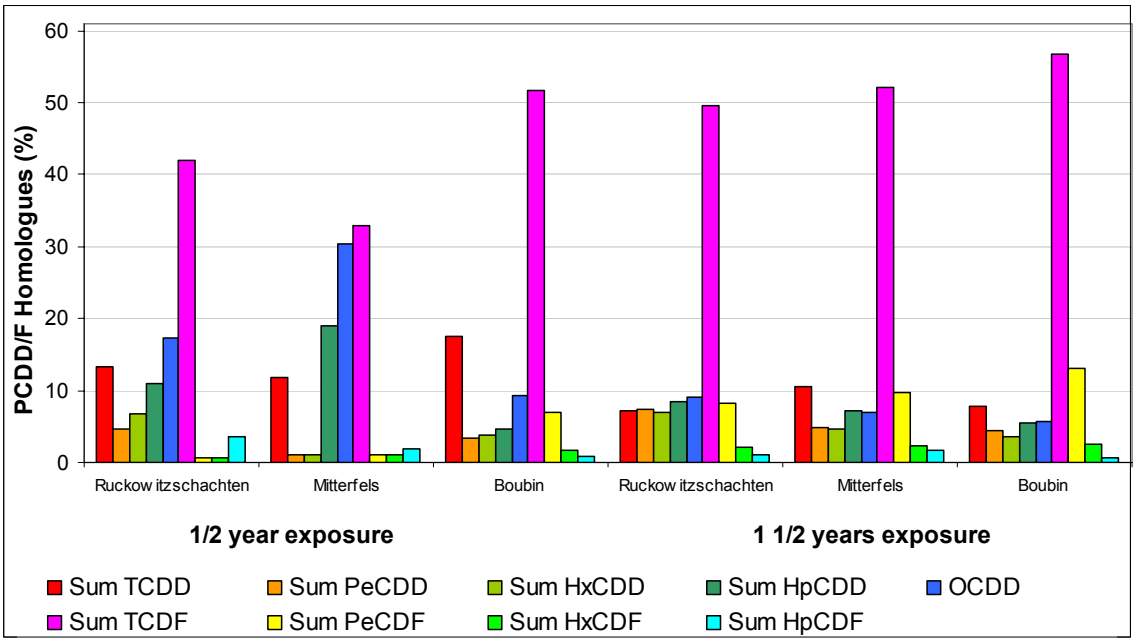


Figure 3-19: PCDD/F homologue concentrations in SPMD ½ year and 1 ½ years exposure at 3 sites. Samplings in October 2003 and 2004.

3.4 SPMD and CSB as passive samplers for air monitoring of POP

3.4.1 OCP

The pesticides in CSB showed different accumulation behaviour regarding exposure time. A first group is defined by the α,γ-HCH isomers, compounds accumulated to the same concentrations after 1/2 and 1 ½ years exposure in CSB (Table 3-10). A second group conformed by PeCB and HCB showed lower CSB concentrations at the longer 1 ½ years exposure period than after ½ year exposure. The third group is represented by all other pesticides, which presented higher accumulation at the longer period of exposure. This points out that the PDMS coating is still acting as an infinite sink for this third group. The average air concentrations for PeCB and HCB determined by active sampling were lower when CSB were sampled after 1 ½ year exposure. This could cause the lower CSB concentrations in spite

of the longer period of exposure. However, this fact alone can not explain the observed behaviour since compounds of the other groups, e. g. air concentrations of γ-HCH and p-p´-DDE also decreased in air significantly when both active campaigns are compared (see Campaigns A and E in Table 3-10) but not their accumulation in coated bars. Focusing on the compound properties (Xiao *et al*., 2004; Shen and Wania, 2005) the K_{oa} and octanol-solubility (S_o) are lower for PeCB, HCB and the α,γ-HCH isomers than for the others OCP analysed. This could imply that an equilibrium stage or saturation in CSB is achieved more rapidly during the exposure period for these compounds. Pesticide concentrations in SPMD exposed the same period of time as the CSB showed a similar, even though less remarkable tendency. A trend to a concentration increase for the α- and γ-HCH isomers (first group) was observed in the longer exposed SPMD. Concentrations of the second group decreased, whereas concentrations of the third set of compounds increased at longer exposure periods as observed in coated bars. In conclusion, the performance of the PDMS-coated bars seems to have a lower capacity than the membrane devices, being this capacity in dependence on the compound solubility to the PDMS coating. On the other hand, both devices are able to detect all the OCP compounds under study in both exposure periods. In summary, the time of deployment has to be chosen taking into account the K_{oa} and S_o of the compounds to monitor. ½ year deployment seems to be more adequate than 1 ½ year deployment to determine compounds of the three groups. When considering compounds of the third group longer exposure allows determining an average concentration value after 1 ½ year deployment. This is not possible for other compounds such as PeCB and HCB due to the deviation from the linear uptake stage in SPMD and saturation in CSB.

3.4.2 PAH

The family of PAH analysed in CSB showed, in general, an increase in their concentrations at longer exposure time. The exceptions were Acenaphthene and Fluorene, where a decrease at their concentrations was observed at longer exposure periods. Analysing the PAH properties such as compound lipophilicity (Alves de Lima Ribeiro *et al*., 2003; Odabasi *et al*., 2006) these compounds present lower K_{oa} than the rest of analysed PAH. This did not explain the contrary tendency obtained for ACL. The compounds AC, FL and ACL with similar log K_{oa} (6.52, 6.90 and 6.34, respectively) and molecular weight exhibited a different concentration behaviour related to exposure time. These three molecules are characterized by two aromatic rings as well as a third pentacyclic ring that in the case of ACL has a double bond. This difference in the molecular structure influences the compound solubility (Martin *et al*., 2007) and thus may determine the different accumulation behaviour obtained for ACL in

comparison to FL and AC. When comparing PAH with OCP, the compounds with lower K_{oa}, like HCB and PeCB, were also those compounds that exhibited a concentration decrease at longer exposure periods as seen for AC and FL.

Figure 3-20 depicts FL and PHE as an example of different accumulation with time of exposure in coated bars. Exhibiting similar air concentrations and being both mainly in the gaseous phase, FL with lower K_{oa} than PHE, is not sequestered as efficient as PHE. This effect is even more pronounced in SPMD where the accumulated amounts of FL in percentage of the total accumulated PAH are even much lower than in CSB. These percentages are around 7 and 6 % in SPMD and 21 and 10 % in coated bars after ½ and 1 ½ year exposure, respectively.

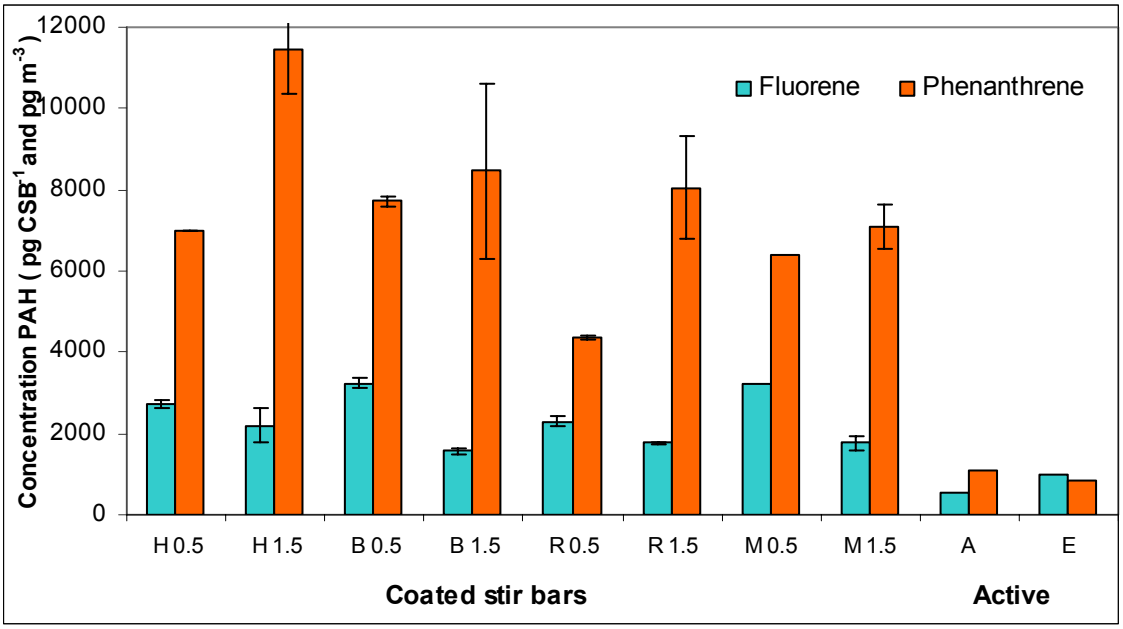


Figure 3-20: Fluorene and Phenanthrene concentrations (pg CSB^{-1}) in coated bars (n=2) after ½ and 1 ½ year exposure at four sampling sites and air concentrations (pg m^{-3} air) at Haidel. 0.5: ½ year exposure, 1.5: 1 ½ years exposure. H: Haidel, B: Boubin, R: Ruckowitzschachten and M: Mitterfels. A and E: Campaigns A and E, respectively.

High molecular weight PAH, in spite of being bound almost completely to airborne particles, were sampled in CSB, not only after 1 ½ years but also after ½ year exposure. Determining the percentage of each compound within the total PAH sequestered in both passive samplers, SPMD were more prone to accumulate high molecular weight PAH than coated bars. There is no correlation between the concentrations achieved in SPMD and CSB neither after ½ year nor 1 ½ years exposure. Accumulation in CSB and 3 months average air concentrations showed also no correlation but the predominant PAH in air (Fluorene, Phenanthrene and Fluoranthene) were also elevated in coated stir bars. FL, PHE and FA represented more than

90 % of the total sequestered amount for CSB with ½ and 1 ½ year exposure. Concentrations sequestered in SPMD after ½ year exposure and air concentrations presented a high correlation such as it was already seen in Figure 3-14.

In summary, due to the broad range of properties such as K_{oa}, vapour pressure and gas-particle coefficient partition of PAH, a commitment for the PAH family as a whole has to be considered when choosing deployment time and passive sampling device. The use of PRC can be helpful to differentiate the uptake stages for each monitored compound in the pooled sample.

3.4.3 PCB

All studied PCB were detected in SPMD devices with the exception of PCB 169 that was neither detected at ½ nor after 1 ½ years exposure. CSB sampled all PCB with the exception of 169 and 126, congeners with the highest toxicological potency regarding PCB. It is important to mention that the PCB 126 and PCB 169 were detected in the average air concentrations in the first period and PCB 126 in the second active sampling period (Table 3-11). The sequestered amounts of PCB in CSB increased with exposure time for all the analysed compounds. However, PCB accumulated in SPMD display different patterns. The congeners PCB 28, PCB 52 and PCB 101 decreased their concentrations at longer exposure periods and none of the other analysed congeners reveal a significant accumulation increase at longer exposure. The lower sequestered amounts of the congeners 28, 52 and 101 at longer exposure time are related to two reasons: the achieved equilibrium for these compounds and the lower mean air concentrations of PCB when the longer SPMD deployment was finished. Previous studies have shown that lower chlorinated PCB congeners reach equilibrium after 200 days exposure (Ockenden *et al*., 2001). In the case of higher chlorinated PCB congeners like PCB 167 and PCB 189 the equilibrium may still have not been met. Factors such as higher chlorinated PCB tend to be retained more on particles and have to be desorbed before being available to diffuse through the membrane, and thus may imply more time to achieve the equilibrium. In addition, higher K_{oa} values indicating more accumulation capacity could cause longer exposure times to achieve the equilibrium. It could also be possible that for some compounds such as PCB 101 and PCB 105 despite having the same degree of chlorination, the different molecular substitution structure determine the different behaviour obtained. For instance, air concentrations of PCB 105 decreased but the amounts sequestered in SPMD remained almost constant during the different deployment times, whereas PCB 101 decreased at longer deployments. PCB 101 has two ortho-chlorines, whereas PCB 105 has only one. A study performed by Falconer and Bidleman (1994) showed that within one homologue group,

non and mono-ortho PCB have lower vapour pressures than congeners with more substitutions in the ortho positions. PCB with lower vapour pressures tend to adsorb more to aerial particles, thus PCB 105 (mono-ortho) has less availability than PCB 101 in the gaseous phase to be sequestered by SPMD. Additionally, the K_{oa} and octanol solubility of PCB 105 is higher than those of PCB 101 (Li *et al*., 2003) and this also results in higher fugacity capacity for PCB 105 in the membrane devices (Harner and Bidleman, 1996). Correlations between SPMD of ½ year exposure and air were in the order of r^2=0.81. In summary, the indicator PCB predominant in the air have reached equilibrium in SPMD after 1 ½ years exposure whereas this effect can not be concluded in CSB deployed the same period. In SPMD under equilibrium conditions there is a tendency to membrane control uptake where the sampling rate is a function of the sampler surface (Huckins *et al*., 2006). On the other hand, the first phase of the uptake can be considered linear and proportional to the surface area of the device. When comparing both passive sampling methods after ½ year exposure, a correlation tendency at the four sampling sites (r^2=0.82, r^2=0.86, r^2=0.87, r^2=0.88) is observable as shown in Figure 3-21 for two sampling sites. The correlations between concentrations in both devices after 1 ½ year exposure remain similar (r^2=0.82, r^2=0.84 and r^2=0.89).

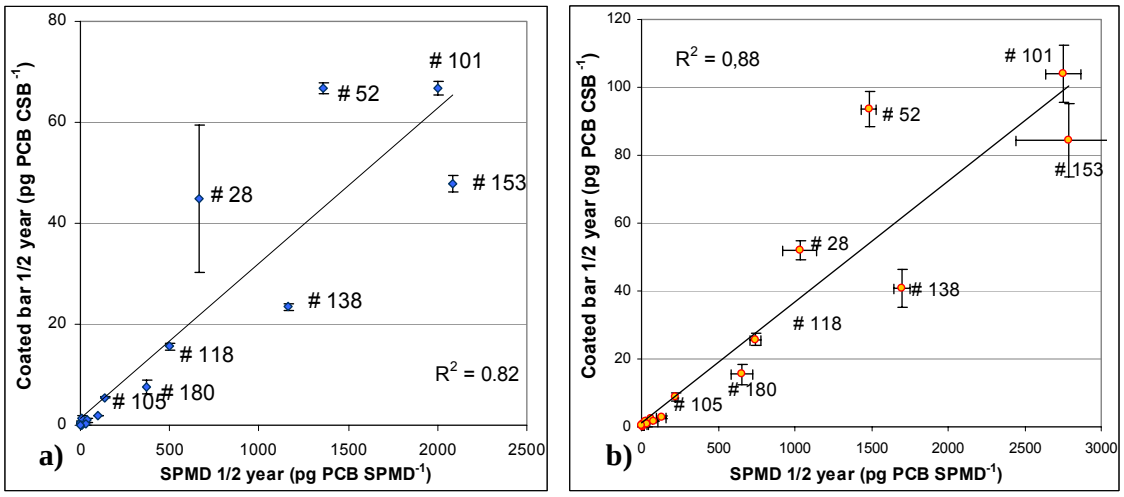


Figure 3-21: PCB concentrations in SPMD (pg $SPMD^{-1}$) (n=2) and CSB (pg CSB^{-1}) (n=2) after ½ year exposure at a) Haidel and b) Boubin. No SPMD repetition available at Haidel.

The congeners PCB 28 and PCB 52 seem to present a better uptake in coated bars than in SPMD after ½ year exposure in relation to the other depicted congeners (Figure 3-21). This could be related to the different uptake stages in SPMD for the PCB congeners. This means that PCB 28 and 52 have already reached equilibrium in SPMD but not in CSB. Therefore, a direct correlation such as shown would not take into account the different kinetics at both passive samplers. Shoeib and Harner, (2002) found that PCB 28 reached the equilibrium in

SPMD within a 3 month deployment and PCB 52 approximately after 1 year deployment. Concentrations of PCB congeners in air and CSB after ½ year deployment present a good correlation (r^2=0.95) (Figure 3-22). Excluding the tri- and tetrachlorobiphenyl congeners PCB 28 and PCB 52, sound correlations between coating stir bars after 1 ½ year deployment and mean air concentrations were also achieved (r^2=0.99) as depicted in Figure 3-22. For these correlations, it was assumed that the TWA concentration of PCB compounds in the studied period were similar to the annual average concentrations. In summary, PCB determined by PDMS coated bars are in agreement with the time averaged air concentrations. Nonetheless, PCB 126, having importance due to its high toxicity, was not detectable by coated bars but in the SPMD after ½ year exposure.

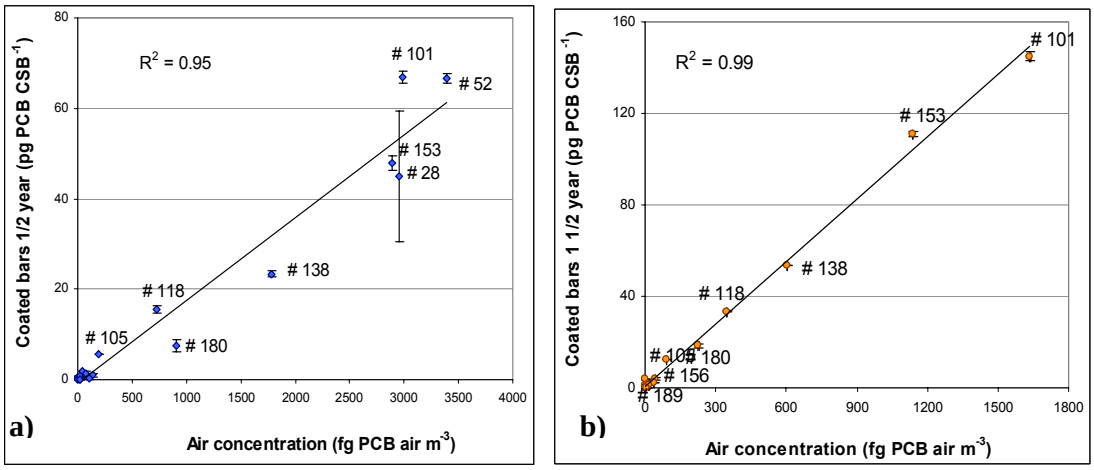


Figure 3-22: PCB concentrations in active sampler and coated stir bars (n=2) after a) ½ year and b) 1 ½ year deployment at Haidel. Note that PCB 28 and 52 were excluded for the 1 ½ exposure correlation.

Table 3-10: Concentration of OCP in SPMD (pg $SPMD^{-1}$) and CSB (pg CSB^{-1}) after ½ and 1 ½ years exposure at four sampling sites. Air concentrations for the A and E campaigns at Haidel are also depicted. SPMD data at Haidel after 1 ½ exposure is not available due to triolein losses. n.d. = not detectable, n. a. = not analysable

Site	***Haidel***	***Boubin***		***Ruckowitz-schachten***		***Mitterfels***		***Haidel***		***Boubin***		***Ruckowitz-schachten***		***Mitterfels***		***Active at Haidel***	
Exposure period	½	½	**1 ½**	½	**1 ½**	½	**1 ½**	½	**1 ½**	½	**1 ½**	½	**1 ½**	½	**1 ½**	**A**	**E**
Compound	**Concentration in SPMD (pg $SPMD^{-1}$)**							**Concentration in CSB (pg CSB^{-1})**								**Air concentration (pg m^{-3})**	
α-HCH	1842	1920	2632	1795	2521	1971	2444	99.1	99.4	129	118	89.4	76.5	90.0	74.9	15.1	14.3
β-HCH	184	201	385	208	374	509	331	1.2	1.7	2.9	3.9	1.5	0.4	1.0	1.3	0.37	0.25
γ-HCH	6444	4984	8434	4720	8382	7102	9586	474	610	596	620	424	397	469	494	34.1	26.2
PeCB	525	589	394	510	438	475	375	50.9	31.1	57.5	32.7	47.2	19.0	49.3	24.8	26.4	24.0
HCB	10282	10896	5012	9972	5066	7925	4906	318	259	384	306	294	181	308	226	65.8	40.3
4-4'-DDT	1106	2077	4849	1978	4533	1430	3328	35.0	84.7	95.9	210	59.1	130	35.6	84.3	1.6	1.0
2-4'-DDT	726	1441	3681	1456	3252	1021	2647	n.a.	31.7	37.9	132	16.7	78.5	1.5	31.9	1.4	0.9
4-4'-DDE	2743	6980	9613	5405	9068	2346	4625	71.6	201	220	500	143	308	82.7	223	4.6	3.1
2-4'-DDE	88.4	191	438	145	384	100	333	2.2	9.5	7.6	22.4	5.4	15.0	2.5	9.7	0.4	0.2
Cis-HCL	445	565	1029	618	1230	513	1143	30.5	77.3	65.2	123	40.8	80.7	35.4	75.1	0.6	0.6
Dieldrin	1401	2127	3495	2060	2970	1732	3005	41.6	116	96.8	228	60.6	173	52.1	127	2.5	2.0
α-Endosulfan	32651	41497	66653	43073	50577	40233	68445	1106	2481	2309	4312	1560	3068	1193	2542	30.3	31.3
β-Endosulfan	4059	6520	15635	62990	12533	5707	15054	92.9	307	196	585	155	516	98	228	2.4	3.7

Table 3-11: Concentration of PCB in SPMD (pg $SPMD^{-1}$) and CSB (pg CSB^{-1}) after ½ and 1 ½ years exposure at four sampling sites. Air concentrations for the A and E campaigns at Haidel are also depicted. SPMD data at Haidel after 1 ½ exposure is not available due to triolein losses. n.d. = not detectable, n. a. = not analysable.

Site	***Haidel***	***Boubin***		***Ruckowitz-schachten***		***Mitterfels***		***Haidel***		***Boubin***		***Ruckowitz-schachten***		***Mitterfels***		***Active at Haidel***	
Exposure	**½**	**½**	**1 ½**	**½**	**1 ½**	**½**	**1 ½**	**½**	**1 ½**	**½**	**1 ½**	**½**	**1 ½**	**½**	**1 ½**	**A**	**E**
Compound	**Concentration in SPMD (pg $SPMD^{-1}$)**							**Concentration in CSB (pg CSB^{-1})**								**Air concentration (pg m^{-3})**	
PCB #28	662	1033	596	765	439	495	471	44.9	54.9	52.1	96.5	48.7	62.4	36.5	91.3	2.9	2.5
PCB #52	1360	1484	1008	1395	1037	1538	1170	66.7	127.9	93.6	194.4	84.8	136.5	70.1	171.7	3.4	2.4
PCB #101	1998	2751	2188	2771	2155	2393	2030	66.8	144.9	104.0	254.7	104.4	217.7	73.7	210.4	2.9	1.6
PCB #138	1160	1698	1694	1809	1671	1187	1415	23.4	53.6	40.9	99.5	40.1	89.4	25.3	76.8	1.8	0.6
PCB #153	2083	2792	2731	2788	2515	1903	2146	47.9	111	84.4	209	84.2	181	53.2	157	2.9	1.1
PCB #180	367	655	603	613	623	438	509	7.5	18.4	15.5	40.4	13.9	37.8	7.2	28.9	0.9	0.2
PCB #77	96.9	132	108	121	163	108	144	1.8	4.0	2.8	7.7	2.5	6.4	1.9	5.0	0.04	0.04
PCB #81	0.8	7.4	3.7	10.9	6.4	n.d.	4.1	0.5	0.9	0.6	1.6	0.4	1.0	0.5	1.8	n.d.	n.d.
PCB #126	6.6	5.2	6.9	n.d.	19.2	n.d.	12.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.001	0.002
PCB #169	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.006	n.d.
PCB #105	139	219	236	270	238	200	198	5.5	12.2	9.0	22.1	9.0	20.6	6.5	17.9	0.19	0.09
PCB #114	15.6	23.5	24.1	22.1	24.2	9.3	22.8	0.9	1.6	1.5	2.7	1.2	2.7	1.0	2.4	0.02	0.01
PCB #118	496	746	780	758	691	660	626	15.6	33.4	25.8	59.5	24.4	48.6	17.1	45.2	0.73	0.34
PCB #123	10.7	61.0	41.3	65.2	40.6	25.5	30.7	1.2	3.8	2.3	5.3	0.8	4.6	0.6	4.0	0.08	n.d.
PCB #156	35.5	82.7	68.8	116.8	54.6	51.3	51.9	1.0	2.2	1.8	5.0	1.8	4.2	0.9	3.9	0.14	0.03
PCB #157	8.1	10.6	5.0	19.8	7.0	8.9	4.4	n.d.	n.d.	0.3	0.3	0.1	0.5	n.d.	0.2	0.02	0.002
PCB #167	27.7	38.6	51.2	73.7	49.1	37.5	44.0	0.3	1.0	0.8	2.9	0.6	2.4	0.4	2.1	0.10	0.016
PCB #189	n.d.	2.9	6.1	n.d.	4.5	n.d.	5.0	0.1	n.d.	0.2	0.2	n.d.	0.4	n.d.	n.d.	0.02	0.004

3.5 Results in the Alpine Region

In this section based on Papers III and V, the SPMD performance is described regarding different periods of deployment and different altitude profiles in the Alpine region. Two sampling campaigns were carried out consecutively: May - November 2005 (Period 1) and December 2005 - May 2006 (Period 2). A third campaign embracing the Period 1 and 2, thus from May 2005 to May 2006 (Period 3) was also performed. Compounds with SPMD blank percentage values higher than 50 % were not considered in the results. Consequently, from all the quantified PAH, the following analysis is based only on the compounds that fulfil this pre-requisite; Phenanthrene (PHE), Fluoranthene (FA) and Pyrene (PY). All the analysed OCP presented satisfactory blank values. Unpublished data regarding the matrices soil and needles are also included.

3.5.1 Monitoring of PAH by SPMD in the alpine region: altitude profiles

PAH: SPMD regarding time and season of deployment

The analyzed PAH, characterized by being mainly in the gaseous phase, are sequestered in higher amounts in the first period than in the second or the third period. Comparing the first and the second ½ year deployment period, higher concentrations are determined when the sampling is conducted in early winter (Period 1). PAH in air in a mountainous spruce stand site were also detected in higher concentrations in winter than in summer as previously discussed in Section 3.2.2 as well as in other studies. These findings are in concordance with the data obtained here. This also means that the SPMD deployed for ½ year give a qualitative pattern of the seasonal air changes at two different annual seasons. In the aim to determine whether a quantitative determination is possible, we compared the sum of the two consecutive 1/2 year SPMD deployments (Period 1 + Period 2) with the sequestered amount determined in the annual SPMD deployment (Period 3) as seen in Figures 3-23, 3-24 and 3-25. The values obtained after 1 year deployment (Period 3) resemble the last ½ year sequestering (Period 2) and therefore, not representative as a mean annual value (blue and green lines, respectively). On the other hand, as the uptake amounts of Period 1 tend to be much higher than Period 2, the sum of both periods (dashed line) resembles Period 1 (red line).

Period 2 and 3 finished in late spring where the mean temperatures are higher in comparison to the end of Period 1. Lower temperatures influence the SPMD-air coefficient partition that is expected to be similar to the octanol-air coefficient partition. The K_{oa} values increase at lower

temperatures and as a consequence the compound affinity within the SPMD is enhanced. Under these conditions the sampling of the first ½ year deployment (Period 1) is conducted, when the highest accumulation of chemicals is detected. 1/2 year later, the SPMD deployed for 1 year present another uptake stage influenced by the temperature changes and the lower air concentrations that results in lower SPMD sequestering as depicted in Figures 3-23, 3-24 and 3-25. This seasonal behaviour was also observed for organochlorine pesticides in SPMD deployed at the same periods.

Previous studies (Gioia *et al.*, 2006) have shown that 2 year deployment periods to determine PAH air concentrations are not advisable but even 1 year deployment is not adequate for determining average mainly gaseous PAH as concluded here. In summary, ½ year deployment is more representative of the air concentrations than longer time exposure for the analyzed PAH.

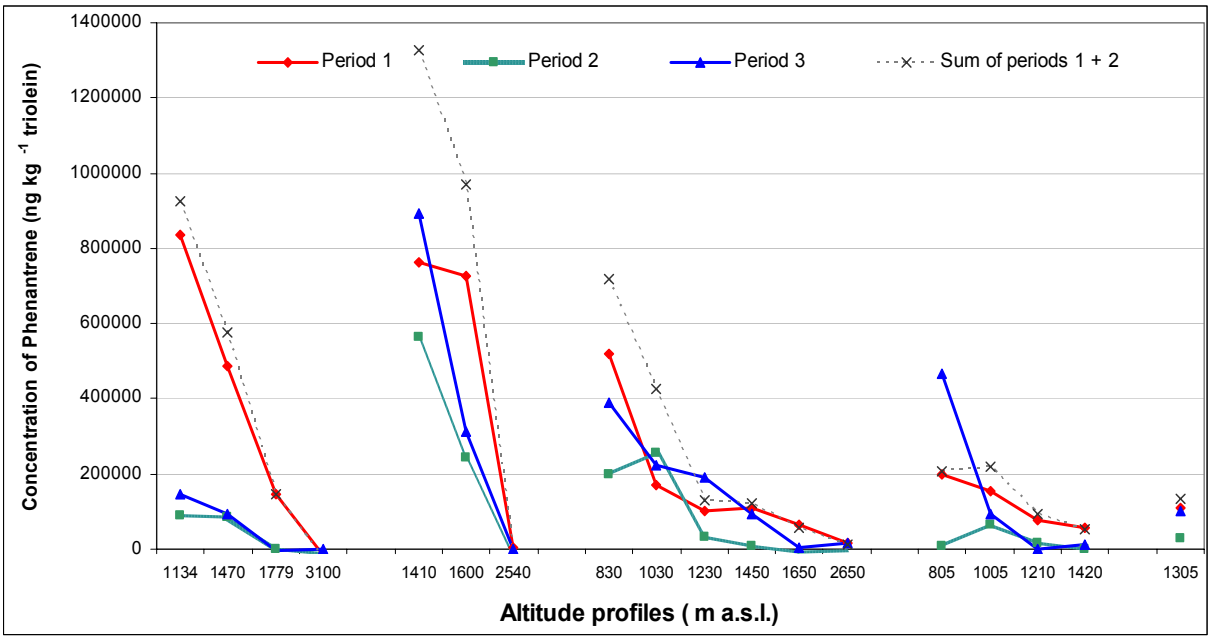


Figure 3-23: Concentration of Phenanthrene (ng PHE kg^{-1} triolein) at 5 sampling sites (4 altitude profiles).

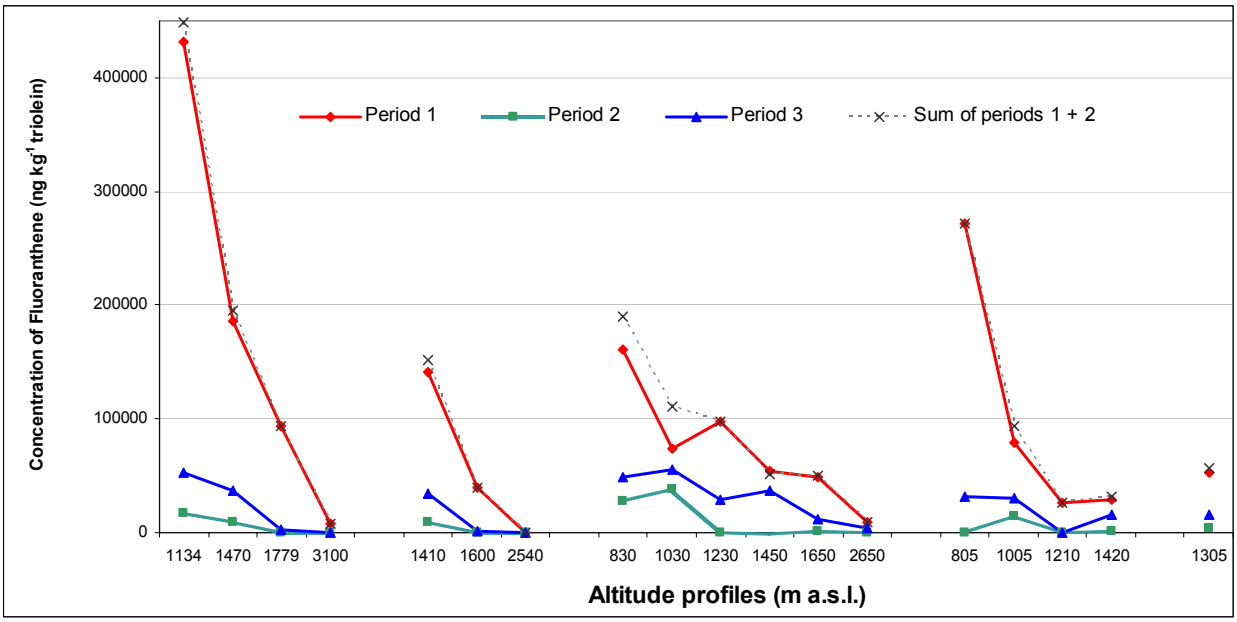


Figure 3-24: Concentration of Fluoranthene (ng FL kg^{-1} triolein) at 5 sampling sites (4 altitude profiles).

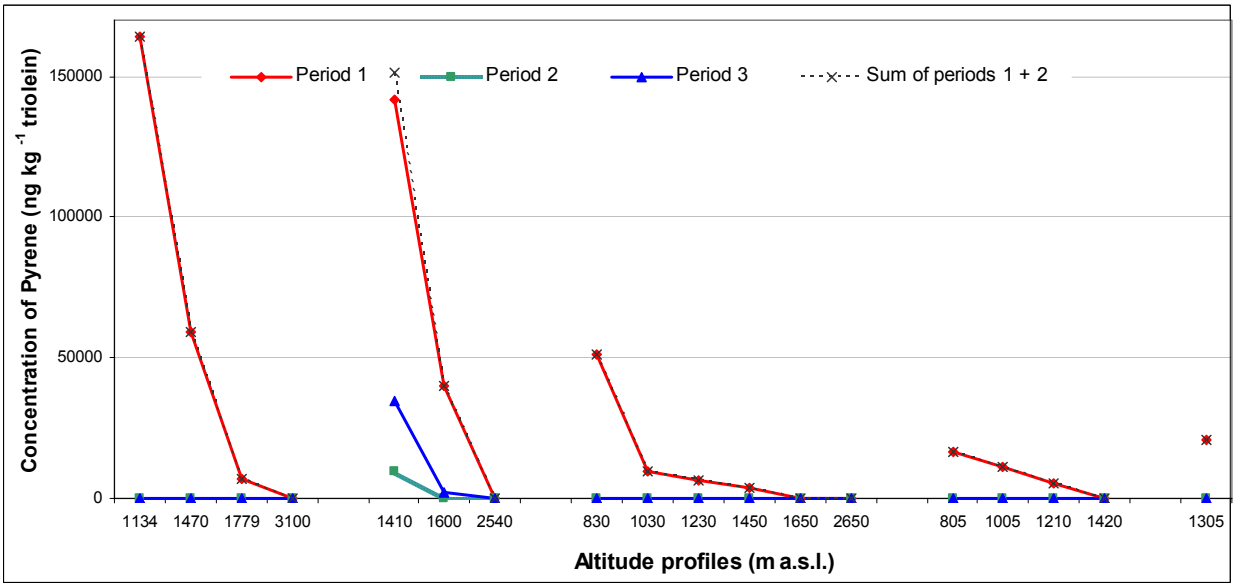


Figure 3-25: Concentration of Pyrene (ng PY kg^{-1} triolein) at 5 sampling sites (4 altitude profiles).

PAH: SPMD regarding altitude profiles

At higher altitudes Phenanthrene, Fluoranthene and Pyrene concentrations decrease until being not detected in SPMD at altitudes higher than 1400 m a.s.l. in the Periods 2 and 3 and at approximately altitudes higher than 1600 m a.s.l. for Period 1. This tendency along the altitude is opposite to that determined for pesticides. PAH can undergo wet and dry deposition

as well as photodegradation which can cause the faster elimination from the atmosphere of these compounds resulting in a strong decrease with increasing distance from potential primary sources (energy production and traffic) at the bottom of the alpine valleys. Studies performed by Kiss *et al*. (1998), showed that at lower temperatures PAH tend to shift their abundance in air from the gaseous to the particulate phase. The deposition increases at larger particle size distribution and dry deposition is favored in presence of these particles, hindering the atmospheric transport along the altitude profile. This could only in part explain the enhanced decrease in PAH concentrations in summer to winter (Period 1) in comparison to winter to summer periods (Period 2) along the altitude profiles (Figures 3-23, 3-24 and 3-25) because PHE, FA and PY, are mainly in the gaseous phase, exhibiting on average 1.9, 19.1 and 29.6 % in the particulate phase, respectively. One important factor to mention is that the inversion layer occurrences are more frequent in winter, hindering the PAH atmospheric transport, particularly in the season of higher PAH generation.

In conclusion, the analysed PAH decreased their concentrations along all the altitude profiles and higher amounts of PAH are sequestered in Period 1, coincidently with the higher PAH production at the bottom of the valleys. SPMD deployments longer than ½ year are not advisable, at least not for PAH that are mainly in the gaseous phase. Seasonal differences play an important role in the uptake of SPMD due to temperature changes and to changes in the physicochemical properties such as the gas-particle distribution for PAH. The fact that the PAH production varies considerably during the different seasons can not be disregarded. Due to this, SPMD deployments considering just the season (approximately 4 to 6 months) are recommended to determine accurately the average air concentrations of the current analysed PAH compounds.

3.5.2 SPMD: Data interpretation regarding exposure time for OCP

All analysed compounds present higher accumulation in the Period 1 than in the Period 2 (Table 3-12). Both periods correspond to ½ year exposure but different seasons at the end of the sampling. The higher sequestering in Period 1 is related to the lower temperatures governing the end of the exposure period in comparison with the temperatures at the end of Period 2. Lower temperatures (but > -4 °C) cause an increase in K_{oa} values, implying a higher compound affinity for the triolein, which explains the higher sequestering in Period 1. This effect, favours the accumulation of compounds, acting the triolein phase as an infinite sink.

As a consequence, SPMD exposure in Period 1 finishes in winter when the highest sequestering of compound occurs. The OCP do not exhibit more atmospheric input in winter as the PAH family did. In this way, the seasonal differences can be attributed to different environmental conditions that influence the sampling uptake. To exemplify this, Figure 3-26 shows the PCA for OCP in SPMD in the different periods. The periods of deployment are clearly separated in the diagram where higher loadings in Period 1 (red circle) in comparison to Period 2 (green circle) are found.

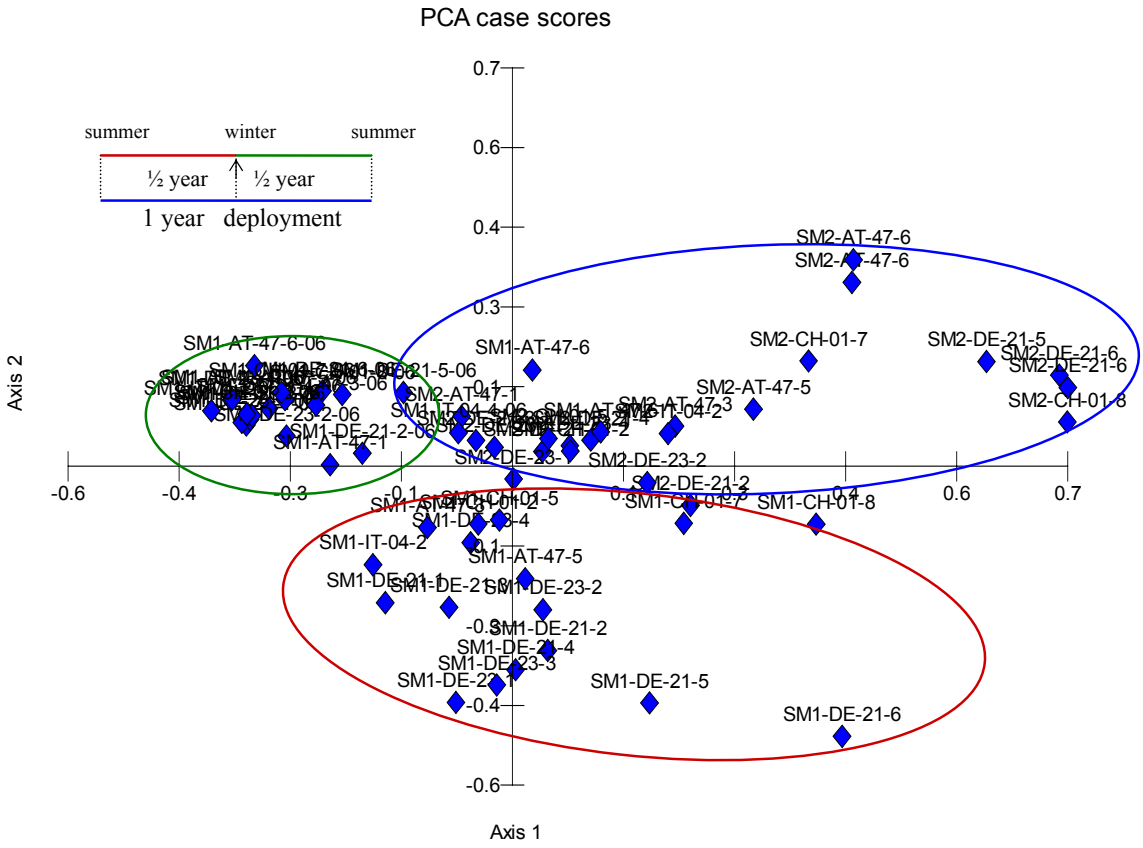


Figure 3-26: PCA case scores (58) of OCP (23). Axis 1 accounts for 43.8 % and Axis 2 for 21.0 % of the variability (64.8 % of the total). SM1: SPMD deployed ½ year, SM2: SPMD deployed 1 year.

The highest loading of the annual Period 3 (blue circle, shifted to the right) is observed in comparison to the 6 month deployment periods. Consequently, as a general trend, more accumulation of compounds occurs in the extended deployment period. Furthermore, higher loading is detected at higher points of the altitude profile, which is shown by the displacement of samples with higher subplot number (higher altitude sites in the profile) to the right.

Analyzing the results obtained at the altitude profiles for both ½ year deployment periods (Period 1 and 2), a similar pattern is observed for each altitude profile for the compounds o,p´-DDT, p,p´-DDT, and the cyclodiene pesticides cis-chlordane, dieldrin and α- and β-endosulfan. The Period 3 also presents the same pattern at each altitude profile as the Periods 1 and 2 for these specified compounds (Figure 3-27). It is also remarkable that the sum of compounds accumulated in Period 1 and Period 2 approaches very well the amount of these chemicals accumulated in the whole year (Period 3). We can infer for these compounds that the membrane devices are still working as kinetic samplers due to the additivity of the Period 1 and 2 when comparing to Period 3. By use of these passive devices, the total accumulation for these chemicals through the year can be measured in order to obtain an annual average value for them. They have high K_{oa} values (K_{oa} >8 at 5° C) that determine large affinity for the triolein phase and also lower vapour pressure in comparison to other pesticides such as PeCB and HCB. The accumulation of these compounds is also characterized by a tendency to higher values at heights above 1400 m a.s.l. in the altitude profile. As an example, the altitude profiles for p,p´ DDT and dieldrin are plotted in Figure 3-27.

In the case of p,p´-DDE the Period 3 assembles the pattern of Period 2 where the accumulation of compounds in the Period 3 is even lower than the accumulation in Period 1. o,p´-DDE shows an intermediate behaviour between the DDT isomers and p,p´-DDE; the profile patterns are similar for periods 1, 2 and 3 but the amount of o,p´-DDE accumulated in the annual period is lower than the sum of both ½ year exposure periods (Figure 3-28). It may be that DDE compounds are more easily exchanged through the membrane device than e.g. DDT compounds and the elimination rate acquires importance. The passive sampler devices of Periods 2 and 3 are sampled together and a similarity in the pattern of the height profile in these periods is determined. This similarity can be related to the stage of the uptake (curvilinear uptake or proximity to the equilibrium) for DDE compounds in passive samplers of Periods 2 and 3.

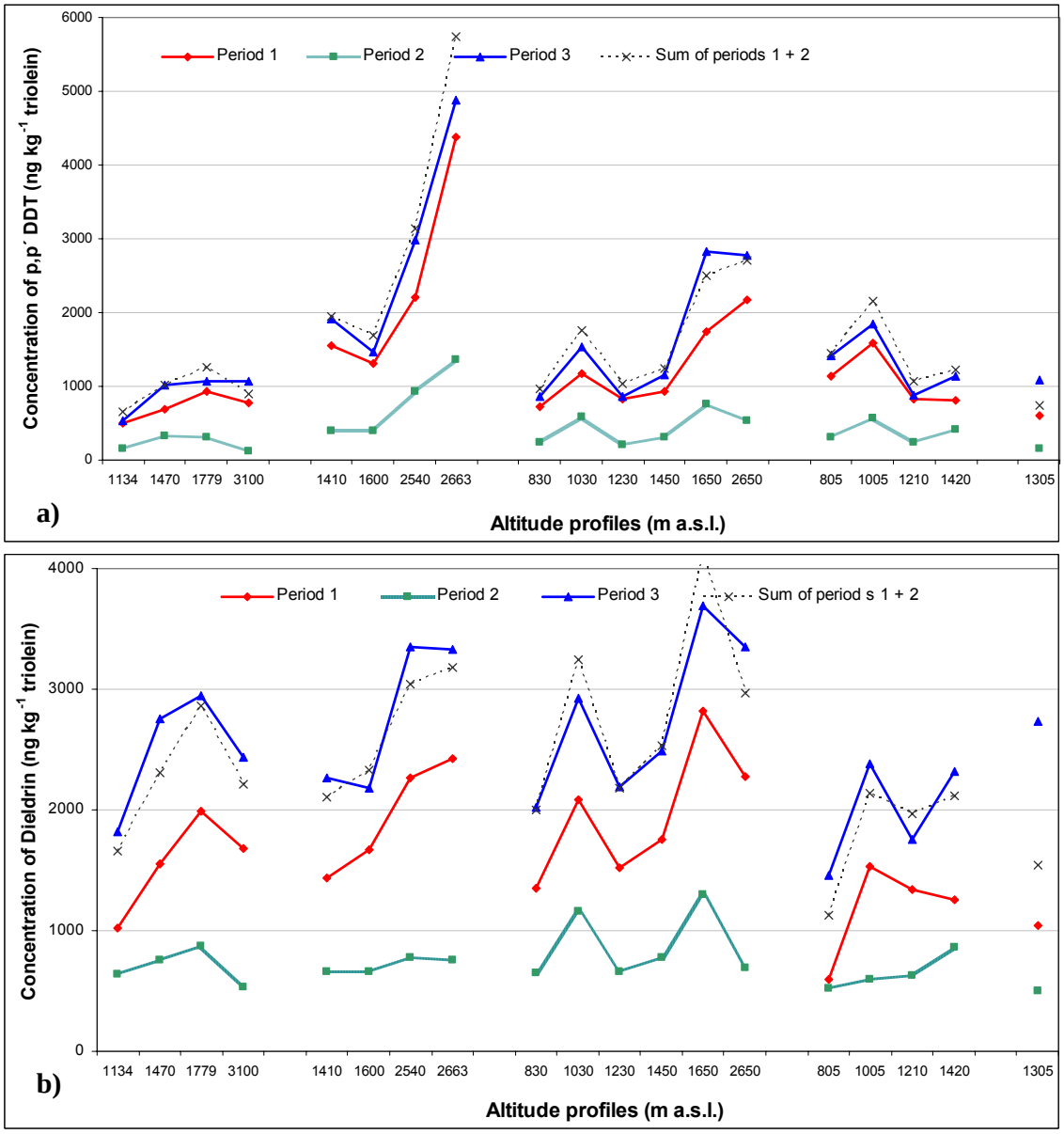


Figure 3-27: a) p,p´-DDT and b) dieldrin concentrations at the altitude profiles for the periods 1, 2 and 3. The sum of periods 1 and 2 is also depicted.

Consequently, the results achieved after one year exposure for p,p´-DDE tend to be similar to the last ½ year exposure (Period 2) in the whole height profile (Figure 3-28). Therefore, we can not consider the obtained accumulation (Period 3) as the annual accumulation for these compounds to estimate average concentrations. Proceeding that way it would underestimate the real concentrations reached at these sampling sites for the DDE isomers.

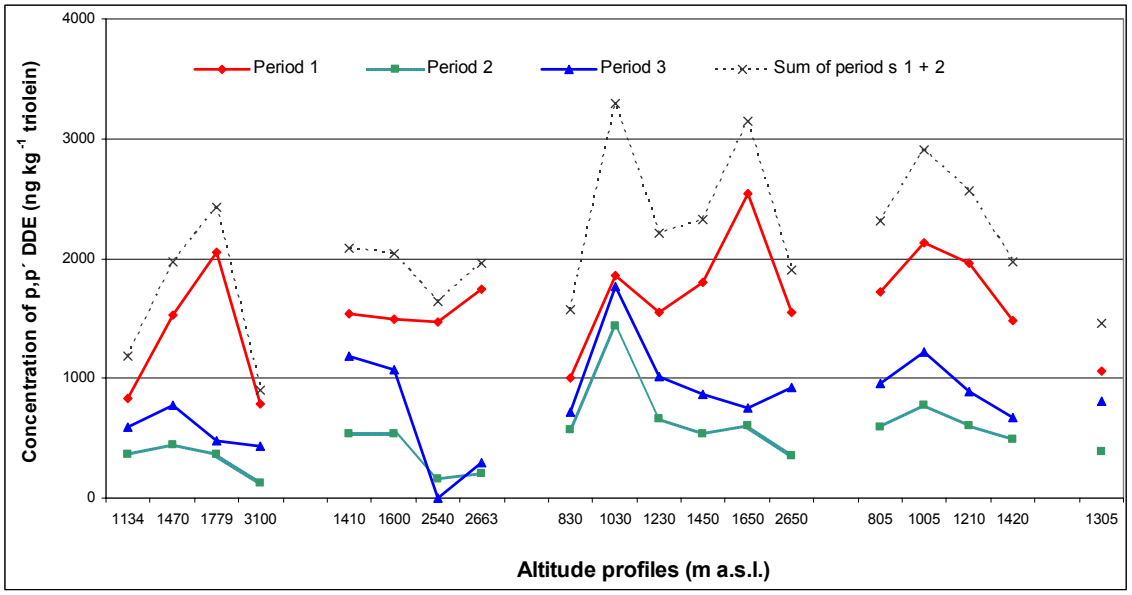


Figure 3-28: p,p´-DDE concentrations at the altitude profiles for the periods 1, 2 and 3. The sum of periods 1 and 2 is also depicted.

PeCB and HCB have similar profile patterns and concentration values for the Periods 2 and 3, as an example HCB is illustrated (Figure 3-29).

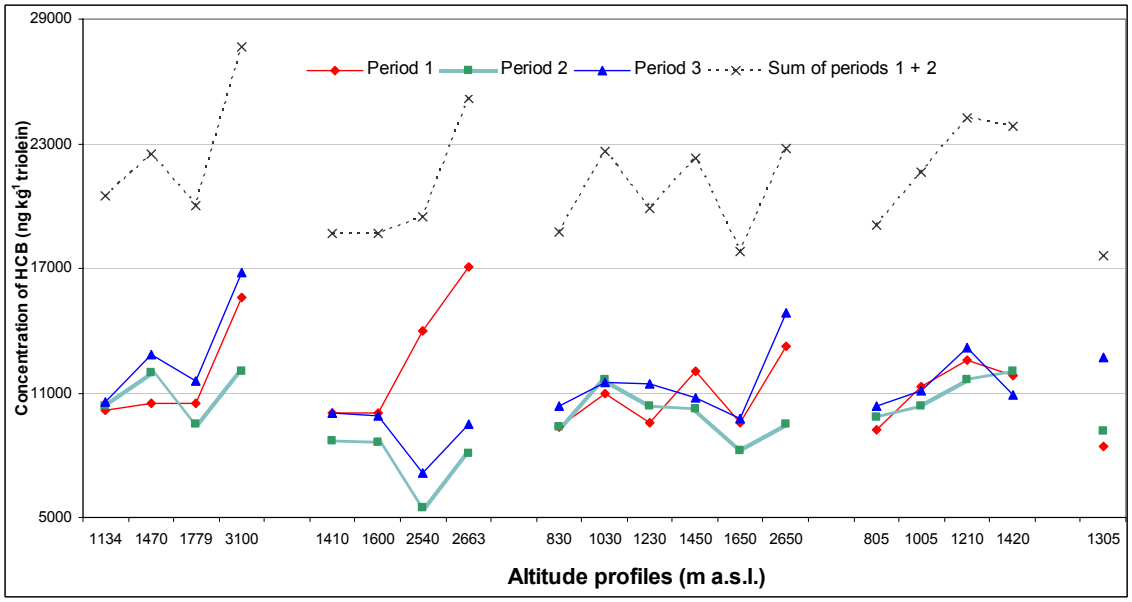


Figure 3-29: HCB concentrations at the altitude profiles for the periods 1, 2 and 3. The sum of periods 1 and 2 is also depicted.

Due to the lower K_{oa} values of these compounds in comparison to DDT and cyclodiene pesticides, a membrane controlled uptake could be expected. Low K_{oa} values increase the

membrane resistance for the mass transfer regarding the overall resistance. This implies that the whole mass transfer process is limited by this step generating a membrane control uptake stage.

Regarding α-HCH and γ-HCH there is no similarity between the patterns of the periods 1, 2 and 3. As a general tendency both isomers decreased in their concentrations in the Period 2 meanwhile the annual period (Period 3) increases significantly at altitudes above 1600 m a.s.l. (Figure 3-30). There is an accumulation increase in the period 3 at higher points of the altitude profile and this accumulation tends to reach the sum of the concentrations in Periods 1 and 2 (above 1600 m a.s.l.). In spite of Period 3 not having pattern similarities with the other periods, it describes the same pattern at both profiles for both isomers; higher SPMD sequestering at higher sites of the altitude profile.

Applying the division for OCP in 3 groups concerning the different uptake stage used in Section 3.4.1, it is confirmed the same grouping at the different seasons and also at the different altitudes. The first group was defined as the α,γ-HCH isomers which accumulated similar amount of compounds after ½ and 1 ½ year deployment in CSB and showed an accumulation increase in SPMD. This accumulation is non linear after 1 year deployment. However, it is remarkable the tendency to the linear uptake stage at higher altitude sites in the profile where the annual accumulation (Period 3) resembles the sum of the other two periods. This could be related to the lower mean temperatures at higher sites (Sites above 1600 m a. s. l.) that favour the uptake and retention of these compounds within the membrane.

Following with this classification, PeCB and HCB (second group), accumulated in SPMD at lower amounts at longer deployment periods are only representative for average air concentrations for periods of ½ year or even shorter. Even ½ year deployments seem to indicate a deviation from the linear uptake stage. The use of Performance Reference Compounds would be useful to determine the current uptake stage for these compounds in SPMD. The third group defined as the rest of OCP are in the linear uptake stage after ½ year deployment but the loss of linearity after 1 year deployment is observable for some compounds, such as DDE, situation discussed above.

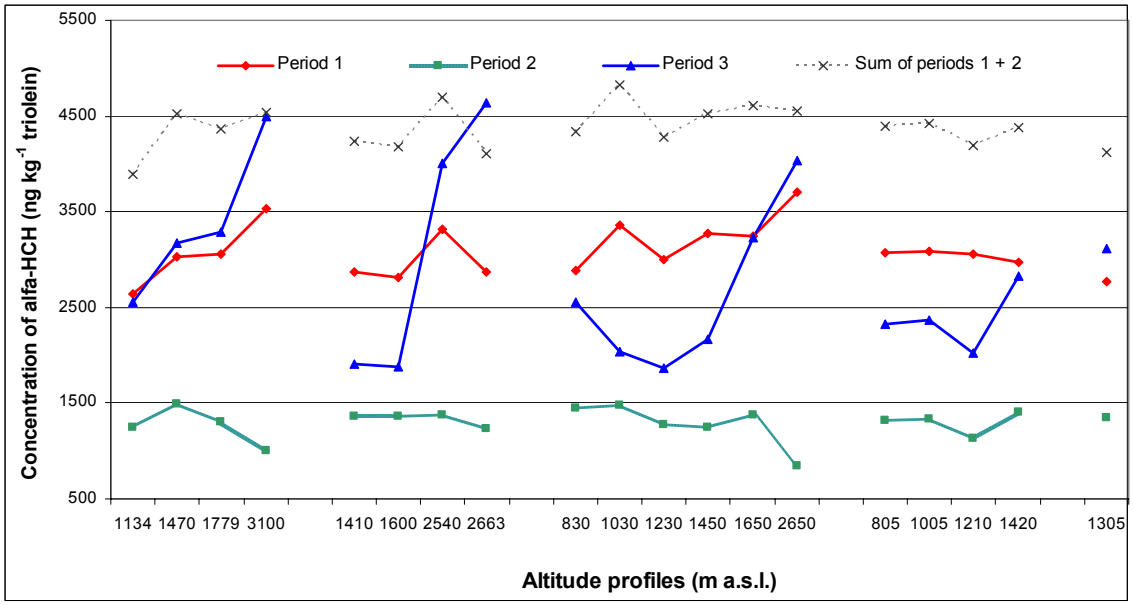


Figure 3-30: α-HCH concentrations at the altitude profiles for the periods 1, 2 and 3.

As a general conclusion, lower temperatures imply more compound accumulation where lower temperatures can be determined by seasonal or altitudinal conditions. In case of compounds that exhibit a steady state in the SPMD, this relationship is direct because the compound accumulation is direct proportional to the K_{oa} of the compound (equation 11, page 25). As higher K_{oa} are observed at lower temperatures for a determined compound, higher compound accumulation is achieved. In case of linear uptake, at lower temperatures the uptake of compounds tends to be membrane controlled. The transient cavities of the LDPE membrane are thermally mediated cavities, decreasing the polymer movement at lower temperatures. As a consequence, the LDPE membrane is the most difficult barrier to overcome in the overall mass transfer process, generating membrane control uptake conditions. Therefore, the general mass transfer equation (equation 5) indicates also an increase of sequestering capacity at lower temperatures. According to the mass transfer coefficient model (equations 4 and 5), more membrane resistance causes higher membrane resistance coefficient (K_m) that generates higher overall resistance (K_o), implying higher total mass coefficient (K_T). As a result, the exponential negative term of equation 5 decreases, generating an increase in the overall equation values, thus higher device accumulation capacities. In the altitudinal gradients, this effect is generated by two factors: lower temperatures and generally, higher wind speeds at the higher altitude sites of the profiles. The highest wind speeds are expected to be at the highest points of the latitude profile, generating

turbulence conditions. Higher turbulence causes a decrease in the thickness of the air boundary layer, decreasing the air boundary layer resistance and favouring a membrane controlled uptake. At this point, it is important to remember that the Stevenson screen boxes where the SPMD are deployed hinder in great extension this wind effect but not completely at the most climatologically exposed points.

Other aspect to consider is that triolein solidifies at -4° C. Environmental temperatures below this point can imply a higher resistance of the triolein in the total mass transfer generated for the different triolein state. The triolein mass transfer resistance was considered depreciable, but this assumption may not be valid below the triolein freezing point. Therefore, temperatures < -4° C do not imply a membrane controlled uptake and consequently a SPMD uptake enhance.

In Table 3-12 is also given the difference in SPMD weight before and after exposure. There is a clear weight increase at the highest altitude sites in particular at Germany (DE-21-6) and at Austria (AT-47-6, data no shown). This could also be related to the different climatologically conditions at the top, that influence the different SPMD uptake. This weight increase could be related with water, spite of the hydrophobic properties of the triolein phase. Compounds with some polarity (hydrophilic-lipophilic balance charge), can attract water in their hydrophilic region and in this way the water can be incorporated together with the organic compound through the membrane to the SPMD, in situations where high atmospheric humidity prevails.

Table 3-12: Concentration of organochlorine compounds (ng organochlorine kg^{-1} triolein) at two altitude profiles. The percentage values indicate the difference between the annual period and the sum of the two half year periods. CH-01: Switzerland-Klosters, DE-21: Germany-Eschenlohe.

Site code	*05.2005 -11.2005 Period 1*		*12.2005-05.2006 Period 2*		*05.2005-05.2006 Period 3*		*Sum of Period 1+ Period 2*		*(Period 1 + Period 2) / Period 3 (%)*		*Altitude (m)*	*Difference in SPMD weight before and after exposure (%)*
	α-HCH	γ-HCH	α-HCH	γ-HCH	α-HCH	γ-HCH	α-HCH	γ-HCH	α-HCH	γ-HCH		Period 1
CH-01-2	2870	4585	1366	2206	1905	1971	4235	6791	45.0	29.0	1410	-0.2
CH-01-5	2815	4188	1362	2200	1881	1600	4178	6389	45.0	25.0	1600	-1.0
CH-01-7	3313	4081	1383	2005	4011	5889	4697	6086	85.4	96.8	2540	2.6
CH-01-8	2874	3962	1230	1859	4631	6174	4104	5822	112.8	106.1	2663	0.5
DE-21-1	2887	7416	1452	4178	2561	6840	4339	11594	59.0	59.0	830	1.6
DE-21-2	3354	7862	1475	4537	2041	4386	4829	12400	42.3	35.4	1030	-0.7
DE-21-3	3001	7022	1282	2989	1865	3033	4283	10012	43.5	30.3	1230	-0.2
DE-21-4	3272	7290	1244	3103	2168	4333	4516	10394	48.0	41.7	1450	-0.1
DE-21-5	3242	6854	1371	3645	3228	6835	4613	10500	70.0	65.1	1650	0.6
DE-21-6	3700	6188	851	1921	4039	9435	4552	8109	88.7	116.3	2650	54.2
	PeCB	o,o´-DDT	PeCB	o,o´-DDT	PeCB	o,o´-DDT	PeCB	o,o´-DDT	PeCB	o,o´-DDT		Period 2
CH-01-2	885	1108	486	377	581	1309	1370	1485	42.4	88.1	1410	0.7
CH-01-5	862	1038	484	376	637	1295	1346	1414	47.3	91.6	1600	0.4
CH-01-7	1256	1828	937	759	1041	2230	2194	2588	47.5	86.2	2540	5.5
CH-01-8	1494	1847	1204	913	1341	2718	2698	2759	49.7	98.5	2663	2.4
DE-21-1	796	577	857	255	949	876	1653	832	57.4	105.2	830	4.7
DE-21-2	869	1056	1330	584	1010	1508	2199	1641	45.9	91.9	1030	0.9
DE-21-3	687	813	869	262	889	895	1556	1075	57.2	83.3	1230	1.0
DE-21-4	767	842	962	353	1125	1274	1729	1195	65.1	106.6	1450	1.3
DE-21-5	632	1624	1131	837	1066	2050	1763	2460	60.4	83.3	1650	2.4
DE-21-6	2374	2003	1886	860	2865	2783	4260	2864	67.3	97.2	2650	27.6
	dieldrin	β-endo	dieldrin	β-endo	dieldrin	β-endo	dieldrin	β-endo	dieldrin	β-endo		Period 3
CH-01-2	1439	2472	664	1339	2271	3586	2103	3811	108.0	94.1	1410	0.8
CH-01-5	1669	2958	663	1335	2185	4137	2331	4293	93.7	96.4	1600	1.3
CH-01-7	2267	5443	775	2347	3356	7631	3041	7790	110.3	98.0	2540	9.4
CH-01-8	2423	5785	754	2463	3332	7191	3177	8248	104.9	87.2	2663	5.1
DE-21-1	1350	2928	645	1162	2023	4259	1995	4089	101.4	104.1	830	7.6
DE-21-2	2080	4802	1164	2622	2925	7817	3245	7424	90.1	105.3	1030	4.5
DE-21-3	1522	4005	661	1548	2190	5158	2183	5553	100.3	92.9	1230	0.1
DE-21-4	1753	4163	778	1767	2491	5886	2531	5929	98.4	99.3	1450	0.4
DE-21-5	2819	8740	1296	3423	3692	12296	4115	12163	89.7	101.1	1650	4.8
DE-21-6	2276	6425	696	3032	3350	10268	2972	9457	112.7	108.6	2650	22.1

3.5.3 Accumulation of OCP in the matrices soil, needles and SPMD in the altitude profiles

In the previous section 3.5.2, a tendency to higher sequestering of OCP at higher elevated sites of profiles was observed in SPMD (Figure 3-26). If PCA is performed with humus results, the same tendency is achieved as seen in figure 3-31.

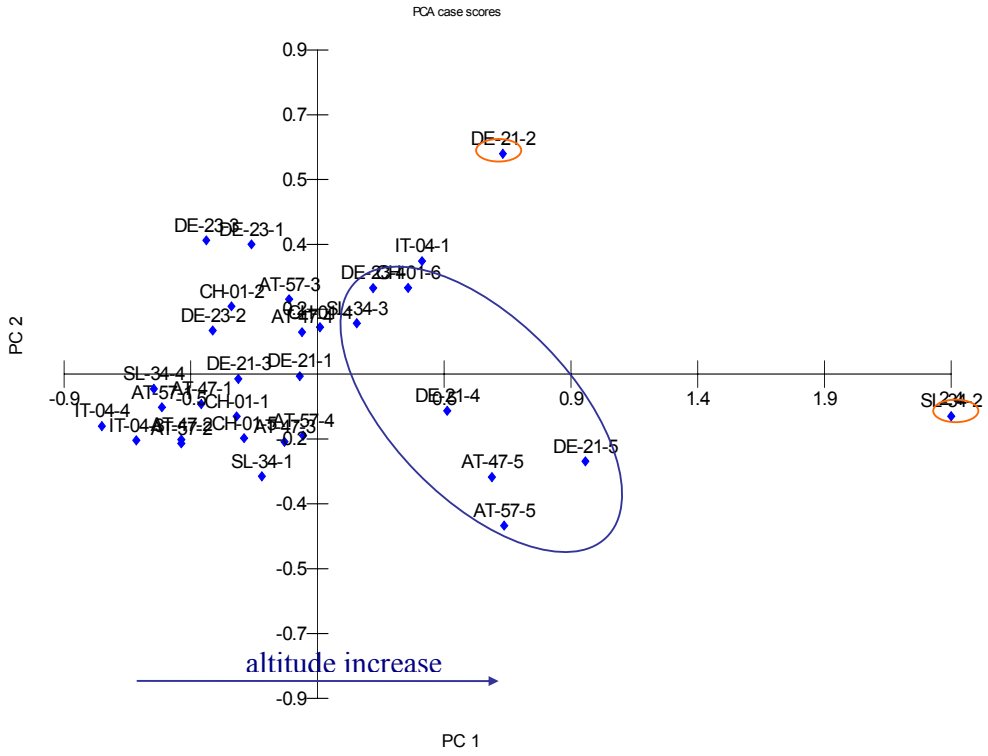


Figure 3-31: PCA case scores (31) of OCP (18). PC 1 accounts for 65.5 % and PC 2 for 9.2 % of the variability (74.7 % of the total). Higher subplots numbers indicate higher sites at the altitude profile.

The blue circle on the right of the graphic embraces the highest altitude sites of the profiles where soil samples were taken. Two outliers are also observed (orange circles). These two sampling sites (DE-21-02: Germany, 1030 m a.s.l. and Sl-34-02: Slovenia, 1354 m a.s.l.) exhibit higher pollutant loading and different distribution of pollutants than the rest of the soil samples. This could indicate punctual sources of OCP pollutants at these two points, thus these sites are not remote sampling points regarding pesticides or the soil matrix is significantly different and no comparable to the rest of the samples. Regarding organic carbon content, nitrogen content and pH, these soil samples are similar to the others (data no shown). Therefore, possible local sources of OCP in these areas, in the present or in the last decade,

have to be checked out. Different geographical characteristics for these altitude profiles could also determine the observed differences.

Performing a cluster analysis with the same humus soil samples, the previous result is confirmed and a new observation can be added (Figure 3-32). The blue circle indicates the higher altitude profile sites, the orange circles, the 2 outliers and the green circle points out a cluster related to samples at an intermediate height (1400 to 1600 m a.s.l.), where also a tendency to different SPMD uptake behaviour was found. The exception within this last mentioned group is the sample IT-04-1.

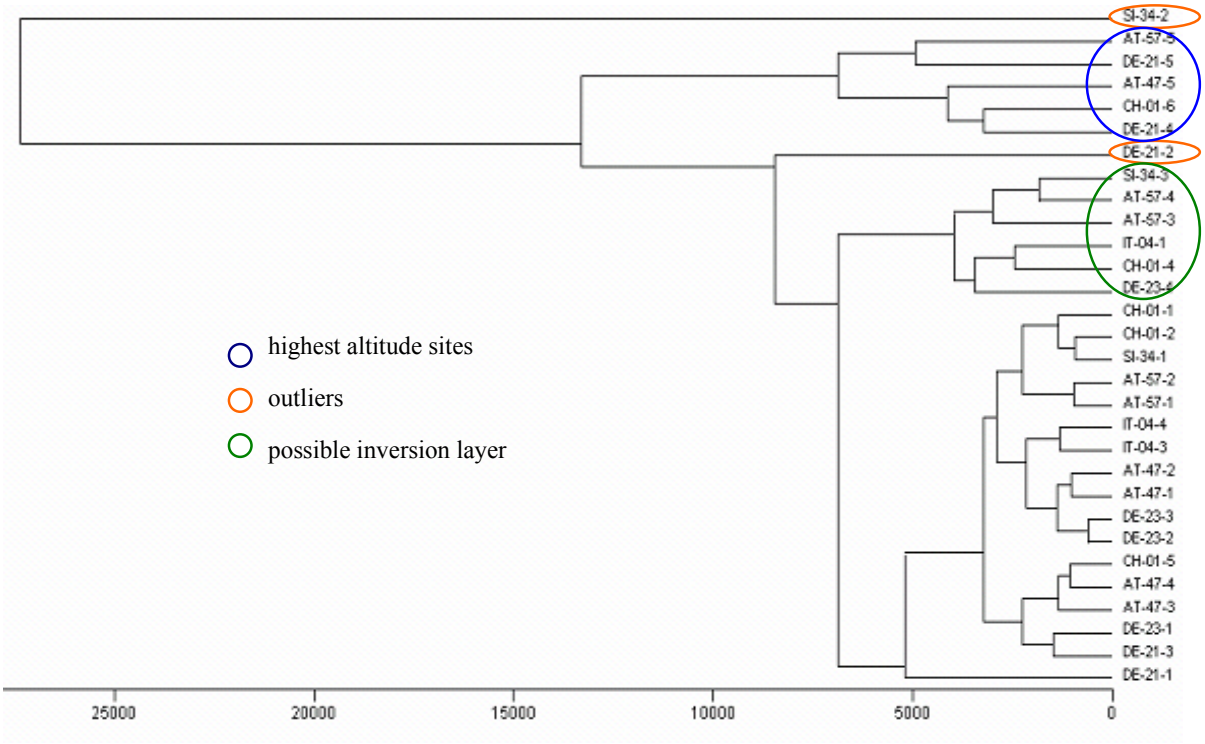


Figure 3-32: Cluster analysis of OCP in humus soil samples (UPGMA). Altitude profiles, sampling 2004.

If OCP concentrations along the altitude profiles where the outliers belong to; are depicted, higher loadings at the outlier sites (DE-21-2 and Sl-34-2) are observed (Figure 3-33). As an example, HCH isomer concentrations are represented, but these higher loadings at these intermediate heights were also observed for other pesticides.

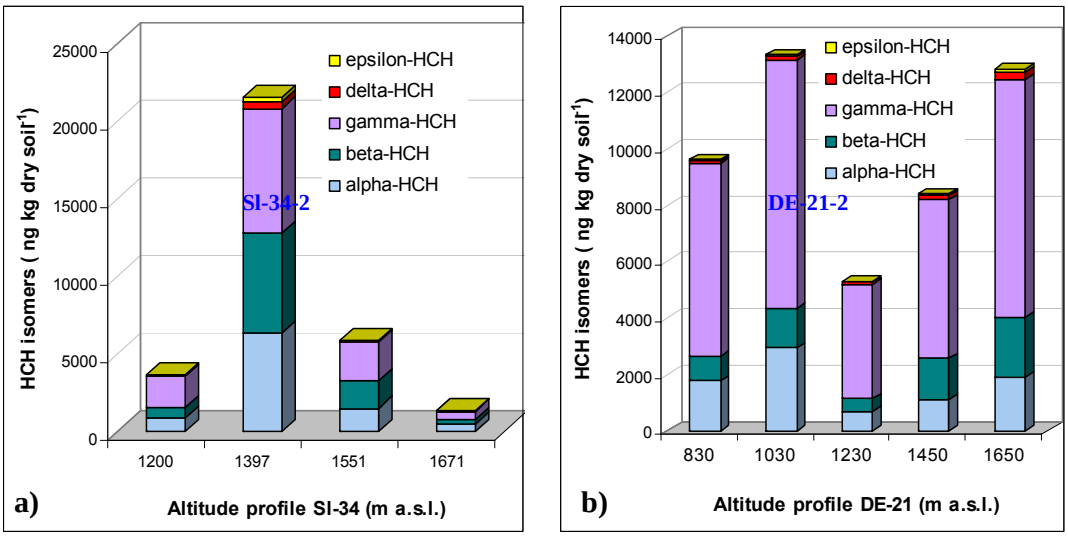


Figure 3-33: Altitude profiles at a) Sl-34 and b) DE-21 sites. Humus sampling 2004.

If results obtained with OCP extracted from the needle matrix are statistically analysed, no cluster pattern is found (Figure 3-34). In this diagram, there is no predominant grouping, but only samples grouped of two, that are close to each other in the same altitude profile. This indicates, that needles are more susceptible to local influences at the time to uptake compounds, hindering the general loading tendencies and distributions found in the matrices soil and SPMD.

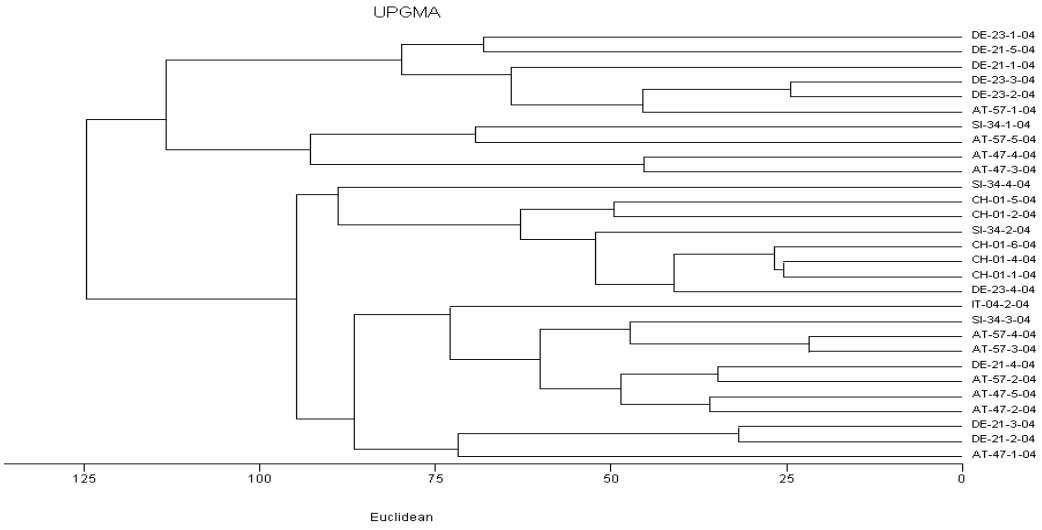


Figure 3-34: Cluster analysis of OCP in needle samples at altitude profiles (UPGMA). Sampling 2004.

3.6 Brief approach to the passive sampling performance

3.6.1 Use of performance reference compounds in SPMD

SPMD, spiked with ^{13}C-PAH and $^{13}C_{12}$-PCDD PRC, were deployed for different periods. The table 3-13 shows the recovery percentage of ^{13}C-PAH PRC in the analysed SPMD after different exposure times. Also included is an unexposed spiked SPMD (control) deployed in a sealed flask and analysed after 404 hours.

Table 3-13: ^{13}C-PAH recovery (%) after different deployment times.

Deployment (hours) / *PRC*	*19*	*44*	*116*	*188*	*404*	*Control* (404 hours)
Anthracene-$^{13}C_6$	77	82	77	68	31	72
Benzo(a)pyrene-$^{13}C_4$	58	62	61	58	62	69
Benz(g,h,i)perylene-$^{13}C_{12}$	73	72	73	72	81	91

Regarding the high molecular weight PAH, ^{13}C-BaP and ^{13}C-BghiP, the amounts of recovery standards are constant during all the deployment periods. Comparing these percentages with the control percentages, there are no noticeable PCR losses for the high molecular weight PAH in the studied period. The low molecular weight PAH ^{13}C-AN, for the analysed period, seems to be adequate. Regarding PCDD-PRC, the lower molecular weight PCDD showed a decrease of 50 % at the end of the exposure period. Surprisingly, the higher chlorinated PCDD were not detectable even after 19 hours exposure (Table 3-14). The results were proven by duplicate analyses.

This fact is in disagreement with the PAH results, considering the higher MW of the Hepta- and HexaCDD in comparison to BaP and BghiP that are not released significantly from the SPMD. Taking into account the higher lipophilicity of PCDD for the triolein phase and their lower volatility no clear explanation has been found to assume that these compounds are released so fast to the environment in comparison to the lower chlorinated PCDD. One possibility is that these compounds undergo indirect photodegradation. These compounds are usually attached to particles in the atmosphere, but in the present experiment these compounds are not sorpted to any surface. This could imply a higher vulnerability to undergo degradation. Photodegradation studies (UV radiation, wave length range 300-400 nm, irradiation intensity 50 W m^{-2}) carried out in different oils with HpCDD demonstrated the fast degradation of this congener (Isosaari *et al*., 2005).

This photodegradation-effect was already suggested by Bartkow *et al.,* (2004) for the PRC anthracene and pyrene in studies performed in the field, even assuming degradation of the

compounds in periods of minutes or hours in case of improper SPMD protection. In the current experiments carried out with ^{13}C-PAH, around 30 % of the total spiked anthracene was still present after 2 weeks exposure.

Table 3-14: ^{13}C-PCDD/F recovery (%) in exposed and unexposed SPMD (n=2) after different deployment times.

Deployment (hours) / *PRC*	*19*	*44*	*116*	*188*	*404*
2,3-DiCDD-$^{13}C_{12}$	74	69	63	65	58
2,3,7-TrCDD-$^{13}C_{12}$	15	14	10	11	16
1,2,3,7,8-PeCDD-$^{13}C_{12}$	6	5	3	4	5
1,2,3,7,8,9-HxCDD-$^{13}C_{12}$	1	1	1	1	1
1,2,3,4,7,8,9-HpCDD-$^{13}C_{12}$	3	3	2	2	2
Control PRC					
2,3-DiCDD-$^{13}C_{12}$	131	103	74	80	93
2,3,7-TrCDD-$^{13}C_{12}$	139	155	144	121	120
1,2,3,7,8-PeCDD-$^{13}C_{12}$	111	106	113	109	113
1,2,3,7,8,9-HxCDD-$^{13}C_{12}$	148	123	137	101	106
1,2,3,4,7,8,9-HpCDD-$^{13}C_{12}$	114	110	102	96	92

The loss of PRC was later also confirmed in the field, where PAH-PRC and PCDD-PRC spiked in SPMD were not found after 6 months deployment. To exemplify this, the PCDD-PRC amounts before and after deployments are given in Table 3-15.

In this field work not only the PRC of the exposed SPMD but even the PRC in SPMD controls, deployed in hermetic flasks simultaneously with the samples, were below to the limit of detection. Only unexposed SPMD controls left at -20°C in the dark during the SPMD deployment kept the PRC standards at their original amounts. Each SPMD was spiked with 465 pg of PRC and the LOD is around 1 pg. Therefore, the obtained results are not influenced by the analytical determinations.

These results lead to the conclusion that the use of PCDD as PRC needs further investigation regarding their fate. The adequate PAH-PRC tend to be in the range of intermediate molecular weight PAH, to obtain a compound release in a range between 20-80 % of the total spiked in order to calculate the sampling rates. The higher molecular weight PAH studied are non adequate, at least in the period of deployment considered. ^{13}C-AN seems to be satisfactory for deployments of approximately 2 weeks.

Table 3-15: ^{13}C-PCDD/F amounts in exposed and unexposed SPMD.

Initial concentration (pg SPMD^{-1})	*$SPMD_{field}$ exposed*	*$SPMD_{field}$ unexposed*	*$SPMD_{-20°C}$ unexposed*
2,3-DiCDD-$^{13}C_{12}$	465	465	465
2,3,7-TrCDD-$^{13}C_{12}$	465	465	465
1,2,3,7,8-PeCDD-$^{13}C_{12}$	465	465	465
1,2,3,7,8,9-HxCDD-$^{13}C_{12}$	465	465	465
1,2,3,4,7,8,9-HpCDD-$^{13}C_{12}$	930	930	930
Final concentration (pg SPMD^{-1})	*$SPMD_{field}$ exposed*	*$SPMD_{field}$ unexposed*	*$SPMD_{-20°C}$ unexposed*
2,3-DiCDD-$^{13}C_{12}$	< 1	< 1	465
2,3,7-TrCDD-$^{13}C_{12}$	< 1	< 1	465
1,2,3,7,8-PeCDD-$^{13}C_{12}$	< 1	< 1	465
1,2,3,7,8,9-HxCDD-$^{13}C_{12}$	< 1	< 1	465
1,2,3,4,7,8,9-HpCDD-$^{13}C_{12}$	< 1	< 1	930

3.6.2 Calculation of sampling rates

Sampling rates were calculated with field data at 2 sampling sites located at the top of altitude profiles (CH-01-8 and AT-47-6). The compounds chosen were those that exhibited the highest concentrations in all the active campaigns and those that were still in the linear uptake. The α- and γ-HCH isomers were included, because as discussed before, they seem to be still in the linear uptake stage at these altitude profile sites. This is a semi-quantitative approach because no PRC were used to perform the calculations. Anyway, the study allows us to analyse the sampling rates in different seasons at 2 different sampling sites, in order to detect possible differences. The sampling rates can be regarded as a theoretic amount of air exchanged in a determined time, where higher values reflect more air exchange capacity of the SPMD. These deployment periods were already described in section 3.5, where higher SPMD accumulation in the Period 1 in comparison with Period 2 was observed for compounds in the linear uptake stage. This is also concordant with the sampling rate values depicted in Table 3-16, where higher sampling rates are observed in Period 1 than in Period 2, being the Period 3 (that embraces the other two periods) an intermediate situation.

Table 3-16: SPMD sampling rates (Nm^3 air day^{-1}) at 2 sampling sites for Periods 1, 2 and 3. N: 1 atm, 273 K.

OCP	*Sampling rates (Nm^3 air day^{-1})*		
CH-01-8 (2663 m a.s.l.)	*Period 1*	*Period 2*	*Period 3*
α-HCH	1.0	0.7	1.0
γ-HCH	1.2	0.8	1.2
p,p'-DDT	9.4	4.3	6.5
o,p'-DDT	6.9	4.9	6.3
p,p'-DDE	5.1	0.8	0.5
o,p'-DDE	6.6	3.5	3.2
trans-chlordane	3.7	2.3	2.5
cis-chlordane	4.6	2.7	2.9
Dieldrin	4.1	1.8	3.3
α-endosulfan	7.3	3.4	5.8
β-endosulfan	6.6	3.4	5.8
AT-47-6 (3100 m a.s.l.)	*Period 1*	*Period 2*	*Period 3*
α-HCH	1.5	0.6	1.1
γ-HCH	1.2	0.3	1.0
p,p'-DDT	9.1	1.6	6.9
o,p'-DDT	9.6	4.1	7.3
p,p'-DDE	4.4	0.7	1.1
o,p'-DDE	6.4	3.5	5.1
trans-chlordane	4.4	2.6	3.5
cis-chlordane	5.5	2.8	5.9
Dieldrin	5.0	1.9	4.0
α-endosulfan	8.6	4.4	6.7
β-endosulfan	8.7	4.0	7.3

It is interesting to remark that the sampling rates for each compound remain in a certain range, as to be expected theoretically, independent of the sampling site. In addition, differences between the sampling periods are in tendency higher than the differences among the different sampling sites. This fact, points out the importance of the temperature in the SPMD uptake and the importance of the predominant temperature at the end of the deployment period. Sampling rates depend on the environmental temperature because of its influence on the compound properties but are independent of the compound air concentrations.

In addition to these results, sampling rates calculated at a remote forest site in a ½ year period deployment which includes the warmer season in the year 2003 are shown for SPMD and

coated bars (Table 3-17). Note that these sampling rates were calculated directly with the field values and were not standardised at normal conditions (N) of 1 atm and 273 K.

Table 3-17: SPMD and coated bar sampling rates (m^3 air day^{-1}) at Haidel, ½ year deployment.

Haidel (1160 m a.s.l.)	*Sampling rates (m^3 air day^{-1})*	
Compound	*SPMD (26.05.03-16.10.03)*	*Coated bars (26.05.03-16.10.03)*
α-HCH	0.86	0.05
γ-HCH	1.3	0.10
p,p'-DDT	4.9	0.16
o,p'-DDT	3.6	-
p,p'-DDE	4.2	0.11
o,p'-DDE	1.5	0.04
trans-chlordane	2.6	0.10
cis-chlordane	5.1	0.23
Dieldrin	4.0	0.12
α-endosulfan	7.6	0.26
β-endosulfan	12.1	0.28

The SPMD sampling rates are similar to those obtained at higher sites in mountainous areas. These sampling rates are representative of the summer period and are in tendency higher than those obtained in Period 2 (November 2005-May 2006) that finished in late spring (Table 3-16). For the compounds still expected to be in the linear stage at lower altitude sites, DDT, chlordane, dieldrin and endosulfan, except for this last one, lower sampling rates than in Period 1 (May-November 2005) are determined. It is possible, that in spite of the favoured mass transfer through the membrane at higher temperatures, the lower K_{oa} values also determined by higher temperatures cause lower sampling rates as a predominant effect. Regarding stir bars, the R_s obtained are lower than the rates obtained in SPMD. As the sampling rate is related to the sampler design, this is concordant with the fact of the lower surface area (approximately 50 times) and accumulation volume (around 4.5 times) of coated stir bars in relation to SPMD.

3.6.3 Calculation of fugacity values

Compounds in equilibrium between two compartments exhibit equal fugacity values in them. The compounds HCB and PeCB are supposed to be in steady stage in SPMD after ½ year deployment. Therefore, calculating the fugacity values for these compounds in SPMD and air, this assumption can be evaluated (Table 3-18). The calculation of fugacity values is based on the theory described in Appendix I.

Table 3-18: Fugacity values (Pa) in air and SPMD.

OCP	*Fugacity values (Pa)*		
CH-01-8 (2663 m a.s.l.)	f_{air}	$f_{SPMD\ Period\ 1}$	$f_{SPMD\ Period\ 2}$
HCB	4.4×10^{-10}	1.1×10^{-11}	5.1×10^{-12}
PeCB	2.9×10^{-10}	1.3×10^{-11}	1.1×10^{-11}
AT-47-6 (3100 m a.s.l.)	f_{air}	$f_{SPMD\ Period\ 1}$	$f_{SPMD\ Period\ 2}$
HCB	4.1×10^{-10}	1.0×10^{-11}	7.5×10^{-12}
PeCB	2.9×10^{-10}	1.4×10^{-11}	1.3×10^{-11}
Haidel (1160 m a.s.l.)	f_{air}	$f_{SPMD\ autumn}$	
HCB	5.3×10^{-10}	1.0×10^{-11}	-
PeCB	2.4×10^{-10}	7.3×10^{-12}	-

Although air fugacities are slightly higher than SPMD fugacities, they can be considered equivalent if it is taken into account the errors generated during the environmental sampling, analytical measurements and calculation estimations. Lower SPMD than air fugacity values indicate higher affinity of the compounds to be sequestered in the SPMD. The air fugacity values are very similar for all the analysed sites. This is concordant with the fact that these compounds are wide-spread and homogeneously distributed in the atmosphere. As the air fugacity is a function of the air concentration and compound properties, the similar air concentrations caused the similar air fugacity values obtained. Air fugacities were calculated with the autumn TWA concentrations. In the case of SPMD, the SPMD fugacities were calculated in the Period 1 (May 2005-November 2005) and the Period 2 (November 2005-May 2006). The SPMD fugacities of HCB and PeCB were higher in the Period 1 than in Period 2 indicating the minor feasibility to be released from the triolein phase in the Period 2. In conclusion, fugacity values in SPMD of Period 1 and air are comparable (steady state). In

addition, lower fugacity values when the temperature over the deployment time increases (Period 2) were determined.

3.7 Bioassay Determinations

In this part of the study, the micro-EROD bioassay results are discussed and compared to the analytical determinations (Paper IV). Following this, the results obtained after participating in an inter-laboratory trial to assess and validate the new yeast-based assay are shown. At last, results of environmental samples using both bioassays are interpreted as a final example.

3.7.1 Dioxin-like compounds in soils: Comparison of analytical measurements and bioassay determinations

Forest systems are able to uptake organic lipophilic compounds into the needle compartment (McLachlan and Horstmann, 1998) and these are transferred to the forest litter, being accumulated in the soil (Howsam *et al*., 2001). Due to this, forested mountainous areas act as deposition regulators of POP. This section describes the results of a micro-EROD bioassay conducted in humus and mineral soil samples from the Bavarian forest and the Alpine regions. In addition, the quantitative analysis of PCDD/F, PCB and PAH family of compounds is carried out and set in comparison with the bio-analytical results. The analytical results are given as Toxic Equivalent values (TEQ) according to WHO (1998) in order to compare them with the EROD results calculated as toxicity equivalent values (TE values) ng TCDD kg^{-1}dry soil. Compounds that still elicit a response after 72 hours incubation in the EROD bioassay are defined as persistent and the results are compared with the TEQ-WHO sum of PCDD/F and PCB. After 24 hours incubation the TE value is related to all the compounds able to elicit a response (persistent and non-persistent compounds) and the EROD results are compared with the TEQ-WHO sum of PCDD/F, PCB and also PAH. Finally, it is important to remark, that the toxicological potential of the environmental sample is much more complex than the theoretically defined TEQ values, and thus the following comparison is an approximation. A direct relationship is not necessarily expected due to the potential presence of further compounds of similar agonism and/or antagonism.

Bioassays performed with extracts from needles and SPMD matrices were not able to give a quantifiable response. All humus samples as well as the mineral soil samples elicited a response in the micro-EROD bioassay. As an example, the induction after 24 hours is depicted for two altitudinal profiles in the alpine region (Figure 3-35).

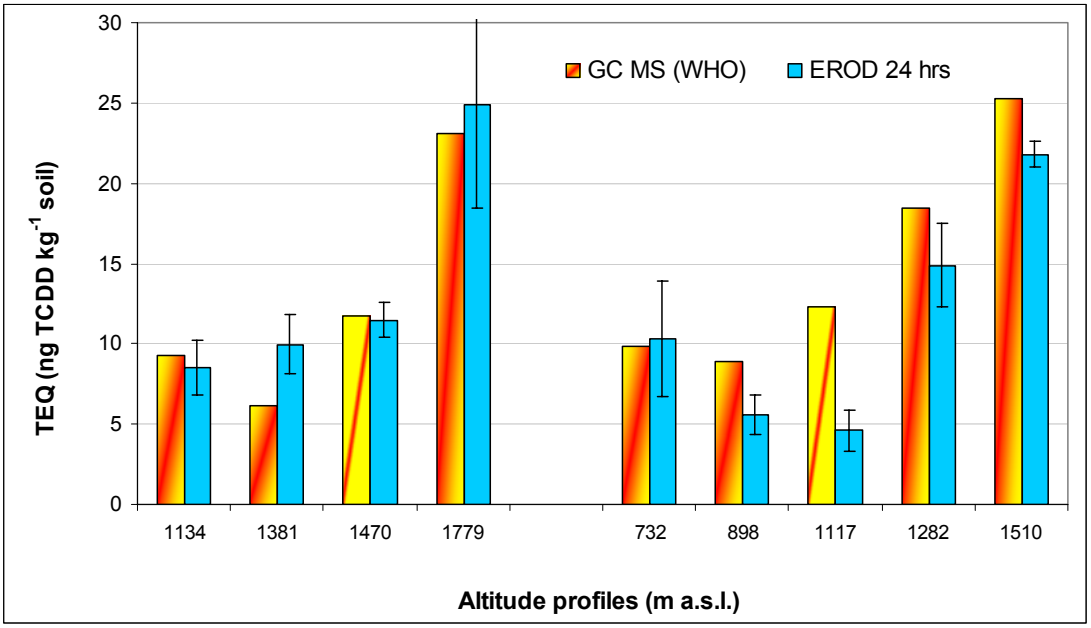


Figure 3-35: TE-EROD values after 24 hours incubation (n=3) and WHO-TEQ values (WHO, 1998) calculated as the sum of PCDD/F, PCB and PAH in humus samples at two altitude profiles.

TEQ values obtained by analytical and bioassay determinations are of the same order. In some cases; the bioassay response is slightly lower than analytical results. This could be caused by antagonists present in the sample and uncertainties in the relative response values in combination with the precisions of the chemical analysis. It is further indicating other or more compounds presented in the sample pool that are influencing in the bioassay. The altitude profiles show a tendency to higher values at higher elevation sites (Figure 3-35), as it was already obtained for pesticides in section 3.5.3.

Comparing EROD results after 24 and 72 hours, the presence of different amounts of non-persistent substances related to the persistent compounds is observed. The altitude profile on the left of Figure 3-36 has a ratio between persistent compounds and compounds able to elicit a response of around 2. On the contrary, the altitude profile on the right is predominant in persistent compounds. This different induction behaviour at 24 and 72 hours indicates the different origin composition of the sample. However, both profiles have concordant TE-EROD values (72 hours induction) and WHO-TEQ values (WHO-TEQ Σ PCDD/F + PCB) within the profile.

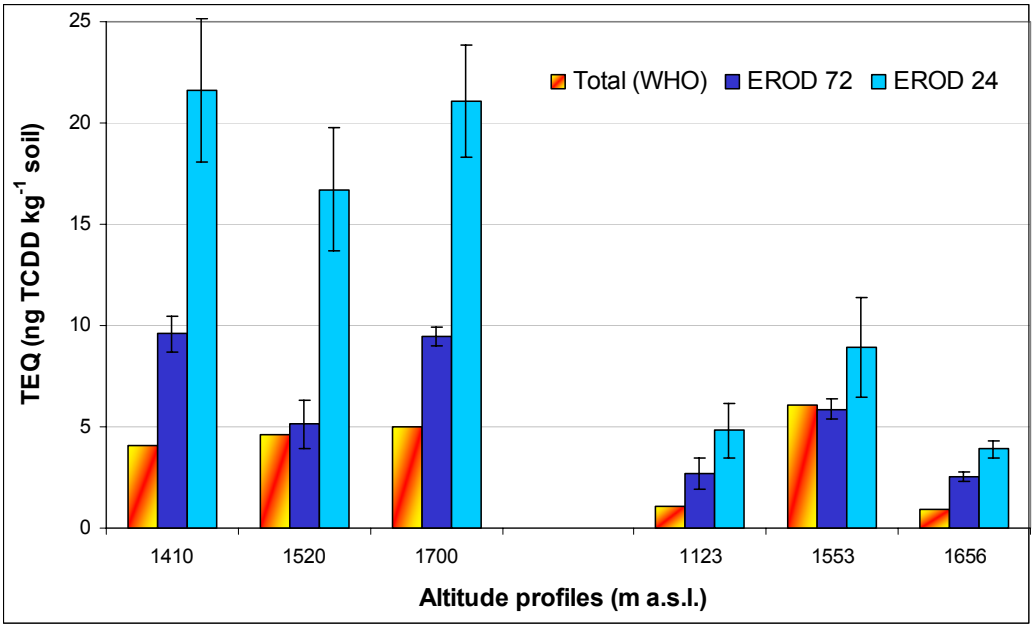


Figure 3-36: TE-EROD values after 24 hours and 72 incubation (n=3) and WHO-TEQ values (WHO, 1998) calculated as the sum of PCDD/F and PCB WHO-TEQ in humus samples at two altitude profiles.

TEQ values of PCDD/F were higher than the TEQ values of PCB in all samples. In Figure 3-37 the results obtained for humus samples in TE values for EROD in comparison to WHO-TEQ calculated for the analytical determinations are plotted. Higher values in the EROD bioassays are expected than in the analytical determinations because the bioassay quantifies the response of the environmental sample as a whole. As a consequence, more chemicals than those calculated by means of the analytical determinations are eliciting response in the bioassay. This is the general trend observed in Figure 3-37 where the majority of the plotted points are found close or above the dividing line that indicates the equivalence for both determinations. TE values of persistent compounds (those that are still present after 72 hours incubation in the bioassay) are similar to the TE values determined by analytical determinations. Therefore, there is not an important loading of unknown compounds in the studied areas and there is major concordance between the bioassay results and the analytical determinations.

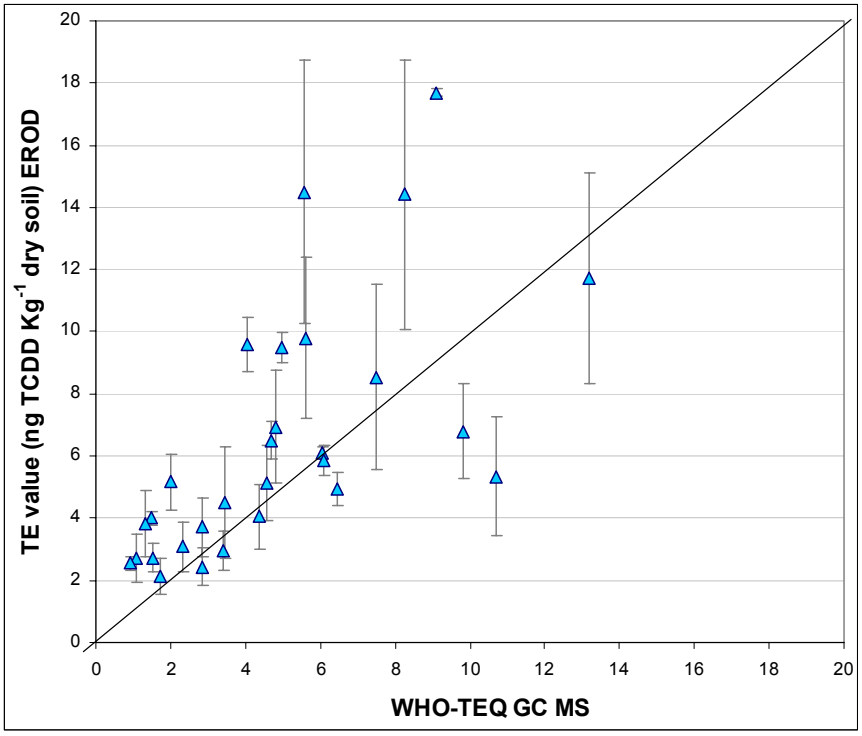


Figure 3-37: TE-EROD values (n=3) after 72 hours incubation and WHO-TEQ values (WHO, 1998) calculated as the sum of PCDD/F and PCB WHO-TEQ from the analytical determinations of 31 humus samples from Norway spruce forest sites.

However, 3 points are clearly located below the line of equivalence. We have to consider that some compounds found in the sample can elicit a slight antagonistic response in the EROD bioassay (Hofmaier, 1999). In this situation, the total induction of the AhR decreases, resulting in lower bioassay activity. These humus samples with higher TEQ values obtained by quantitative determination than in the bioassay are representing three samples with higher concentrations of PCB. PCB are known to act as weak antagonistic substances when they are found at high concentrations (Schlezinger *et al*., 2006). The WHO-TEQ PCB values (ng PCB kg^{-1} dry soil) for these 3 samples are, 4.3, 3.9 and 4.6 in comparison to the mean of the samples 1.7 ± 1.2 (WHO-TEQ ng PCB kg^{-1}dry soil). Analysing the isomer concentration distribution in the samples, higher PCB concentrations in particular for the non-ortho isomers PCB 77 and PCB 126 are observed in these outlying samples. This could be a reason of slightly lower values obtained in the EROD bioassay. Hoffmaier, (1999) analyse the effect of some non-ortho and mono-ortho PCB in the micro-EROD bioassay finding an inhibitory effect in the bioassay activation when these compounds were added with the inducer TCDD. The concentrations of PCB used by Hoffmaier in the EROD bioassay, were of equivalent order than the PCB concentrations here found. Subsequent studies showed that some non-

ortho PCB as PCB 77, PCB 126 and PCB 153 inactivate the cytochrome CYP1A1 and this inactivation is dependent on the PCB concentration (Binelli *et al*., 2006; Schlezinger *et al.*, 2006).

In the case of the humus and mineral soil samples analysed from the Bavarian and Bohemian forests, higher concentrations of PCB and TCDD/F were found. Figure 3-38 shows that the humus samples from these sites with higher PCB concentrations show a tendency to lower TE values in the EROD activity than the total WHO-TEQ of the analytical results.

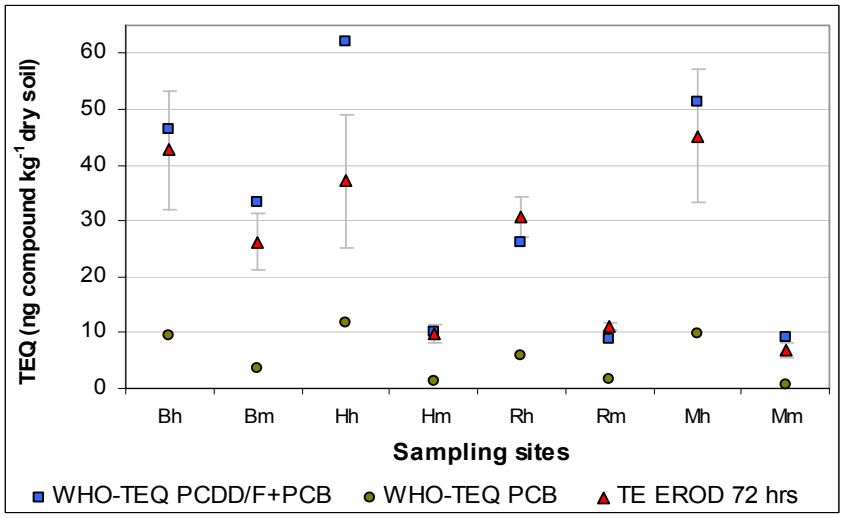


Figure 3-38: TE-EROD values after 72 hours incubation and WHO-TEQ values (WHO, 1998) for PCDD/F and PCB. EROD data points are the mean values of 3 replicates. Site codes: H: Haidel, B: Boubin, R: Ruckowitzschachten and M: Mitterfels. h: humus and m: mineral soil.

A tendency to lower EROD values than TEQ values is observed, especially in samples with relatively high non-ortho PCB to mono-ortho PCB concentrations (See samples Hh and Mh in Figures 3-38 and 3-39). This finding indicates that variations in the amounts of non-ortho PCB could have more influence on the EROD activity, although being at lower concentrations (at least 10 fold less) than mono-ortho PCB. However, it is also probable that the combination of mono- and non-ortho PCB compounds have an influence in the obtained results. Non-ortho PCB have a similar molecular structure (coplanar) as PCDD/F and this implies that this kind of compounds interact more easily in the mechanisms of Ah receptor affinity compared to other PCB congeners. This interaction seems to generate a larger inhibitory effect than the EROD activation generated by other PCB compounds at much higher concentrations.

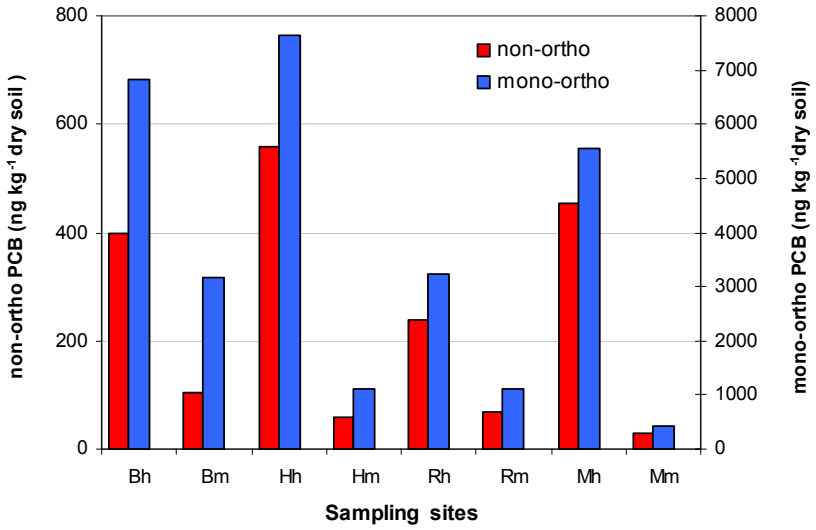


Figure 3-39: non-ortho PCB and mono-ortho PCB concentration values in humus and mineral soils at H: Haidel, B: Boubin, R: Ruckowitzschachten and M: Mitterfels 4. h: humus and m: mineral soil.

To exemplify and reaffirm this, concentrations of PCB 126 and TE-EROD values in humus soils from the Alpine region are plotted. Higher concentrations of this co-planar PCB are associated to lower EROD inductions (Figure 3-40).

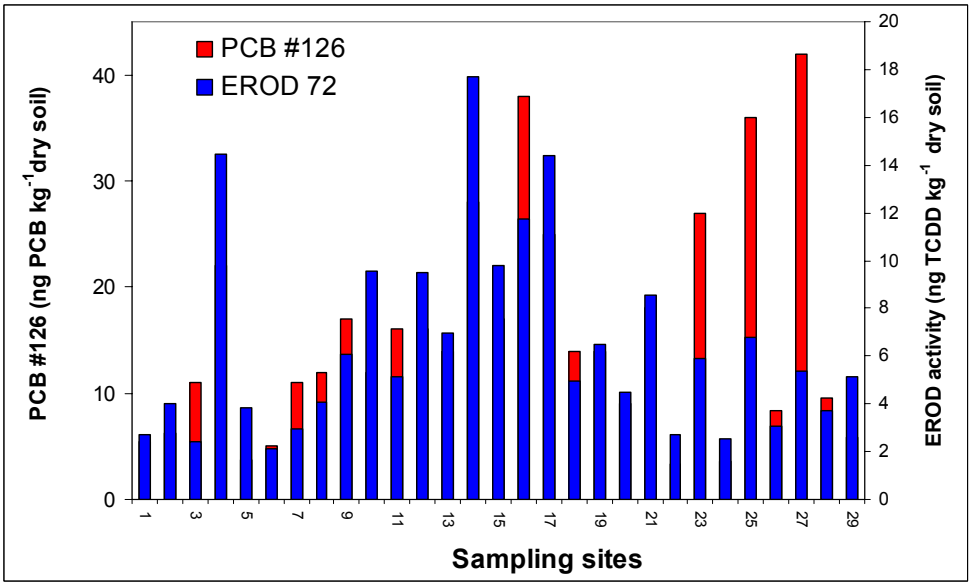


Figure 3-40: Concentrations of PCB 126 and TE-EROD values in 29 humus samples form the alpine region.

In this work, the relationship between analytical and bio-analytical results for persistent compounds is discussed with respect to PCB and PCDD/F present in the sample. Despite of a good correlation between PCDD/F and PCB concentrations and EROD activity, deviations

from the expected results indicate additional factors influencing them. We can infer that the EROD activity decreases at high concentrations of PCB or other antagonists detected in the sample. On the other hand, in remote places far away from sources, the EROD bioassay gives a proper estimation of the overall CYP1A1 induction potential of the samples. The comparison between bio-analytical and analytical results allows us to identify samples where additional contamination with other chemicals is indicated. When the bio-analytical results are lower than the analytical PCB-PCDD/F equivalent results, the presence of high concentrations of PCB compounds or antagonistic modulators could be possible.

3.7.2 Inter-comparison of estrogenicity in water by means of yeast-based assays

The objective of the participation in this inter-laboratory was to determine the suitability, robustness and variability of the new yeast-based bioassay to assess endocrine disrupters in environmental samples. Results obtained at the Institute of Ecological Chemistry are shown together with other participant laboratories which used others recombinant yeast strains and methods for the reference standard 17β-Estradiol 10 nM. This standard was provided as an "unknown sample" to all participating laboratories. Results are the mean value of 6 batches and the results obtained at the Institute of Ecological Chemistry correspond to laboratory 7 (Figure 3-41, red arrow). A good performance in accuracy and precision in the standard measurement was achieved.

Mean values (n=6) of the 11 environmental samples analysed are plotted against the expected values. It is observed again the good accuracy obtained using the new developed bioassay at the Institute of Ecological Chemistry (Figure 3-42). These two graphics were provided after the inter-laboratory report deadline by Dr. Rikke Brix (SWIFT deliverable D23: Inter-comparison of estrogenicity in water determined by yeast-based bioassays). Department of Environmental Chemistry, IIQAB-CSIC, Spain.

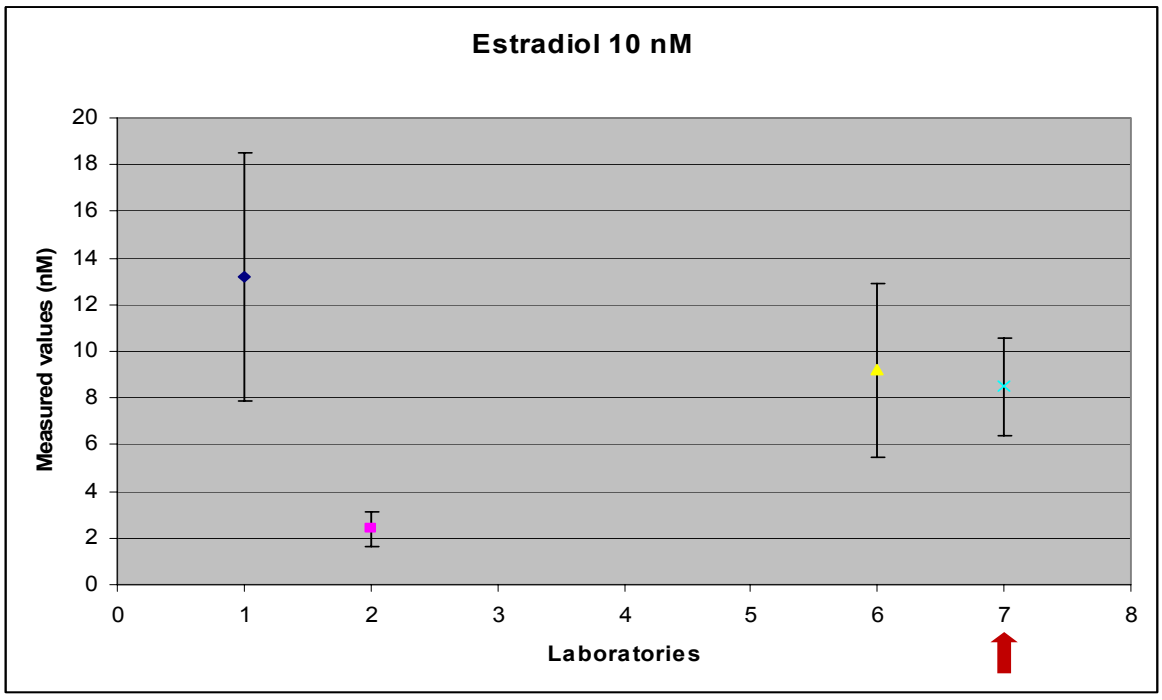


Figure 3-41: Inter-laboratory reported results on the sample 10 nM Estradiol (n=6). Laboratory 7 corresponds to results obtained at the German Research Center of Environmental Health.

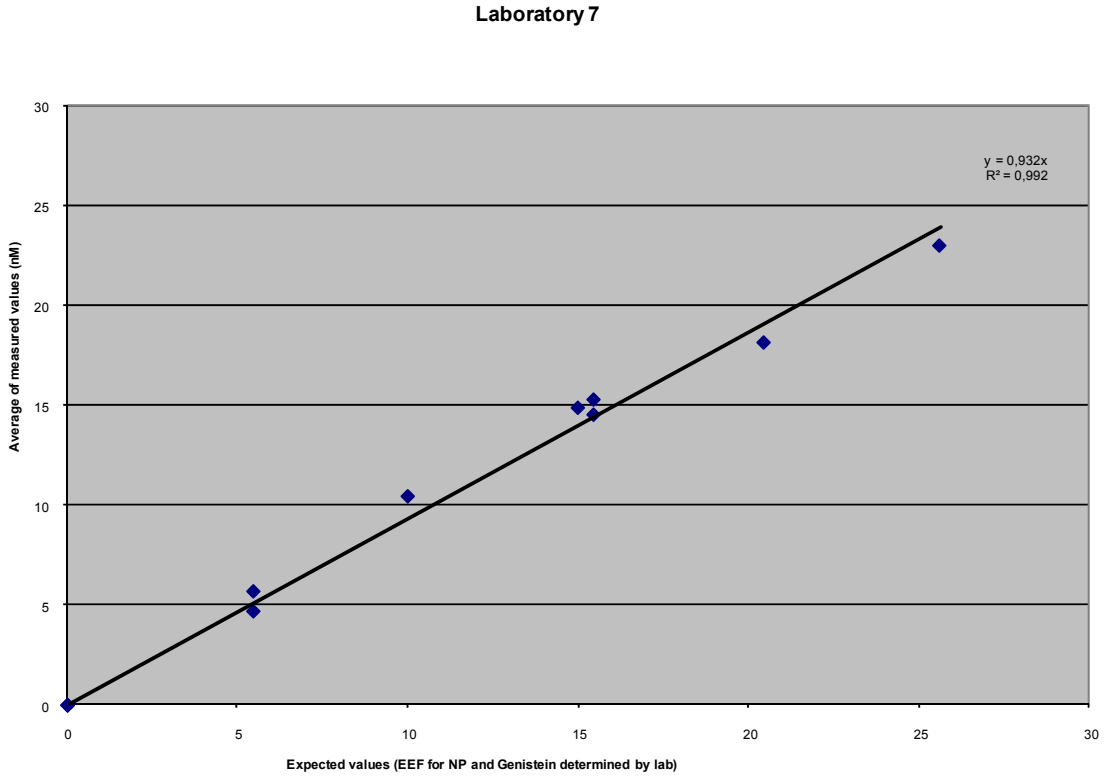


Figure 3-42: Correlation between expected and measured values for laboratory 7.

3.7.3 Determination of the capability of response of the genetically modified yeast in presence of estrogenic substances

In this section, the capability of response of the yeast strain is assessed. To achieve this, the response after performing the bioassay is measured by 2 techniques for 3 potent estrogenic substances at different concentrations. The fluorescence measurement is the classical measurement carried out for this bioassay and the current in use at the laboratory. The flow cytometry measurement is the second technique to study here. The cytometry measurements allow differentiating between inducted and non-inducted cells and therefore, to determine the amount of genetically modified yeast able to give a response in the bioassay. The three estrogenic substances activated more responsive elements at higher standard concentrations following a sigmoid increment (Figure 3-43) and reaching response saturation.

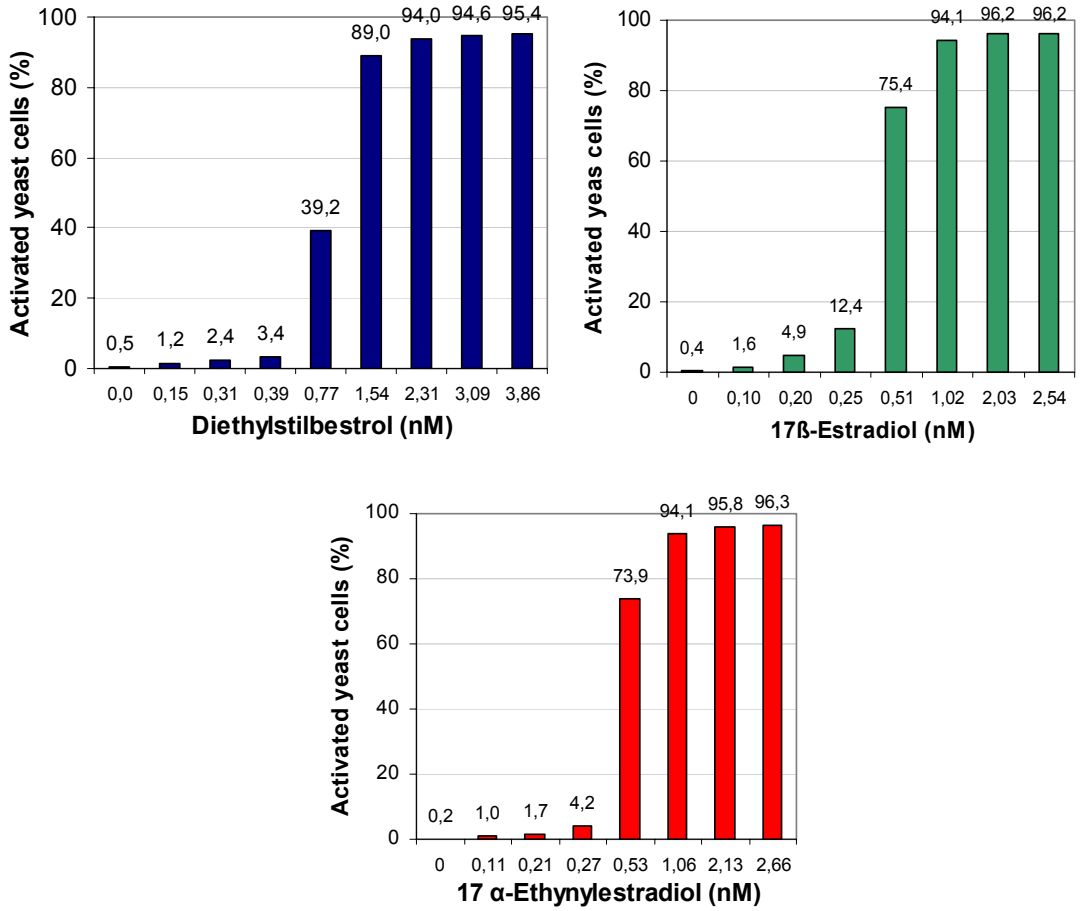


Figure 3-43: Percentage of genetically modified yeast activated after 18 hours incubation at different standard concentrations (nM) for DES, 17ß-E and 17α-E measured by flow cytometry.

For the flow cytometry measurements two populations are defined after excitation; population 1 (P1) is the inactivated population and population 2 (P2) is the activated population. Each measurement included approximately 2 x 10^5 yeast cells. The measurement of fluorescence given for the activated P2 in the total measured population (P1+P2) determines the specific activation of the sample (Fluorescence per 2 x 10^5 cell counts). The background level of excitation after incubation (amount of P2 population in the blank) was up to 0.5 % of the total yeast population and is considered acceptable.

Dose-response curves are calculated by iteration with fluorescence measurements performed in the luminescence multi-well plate reader (Figure 3-44a) and by flow cytometry (Figure 3-44b). The results showed the lower potency of DES in comparison to 17ß-E and 17α-E in both type of measurements. Higher concentrations of DES standards were necessary to achieve similar responses in the dose-response curves to those achieved with 17ß-E and 17α-E standards. Higher sensitivity in the induction is related to lower EC_{50} values and better sensitivity was obtained by flow cytometry as shown in Table 3-19. Lower EC_{50} values also mean higher drug potency. The drug potency for 17ß-E and 17α-E are close enough that the different measurement methods determine different order in their drug potency (Table 3-19). One explanation to this result is the measurement error inherent to the applied methods. Another possible reason is the incubation time. The estrogenic standards have a maximal response that is time dependant and this optimal incubation time is not coincident for all of them. The fluorescence measurements are not only considering the amount of cells that are fluorescing but fluorescence per well simultaneously for the 3 substances in the 96 well plate. As a consequence, it is possible that the response decreases for 17ß-E because of the shift from the optimal read out. Therefore, the slope of the 17ß-E has lost steepness due to the loss of response as seen in Figure 3-44a. Further studies at different incubation times would be necessary to verify these results.

Table 3-19: EC_{50} values for dose-response curves after 18 hours incubation for DES, 17ß-E and 17α-E (nM). Response determined as fluorescence per well (n=6) and activated cells in the total cell population.

Compound	*DES*	*17ß-E*	*17α-E*
EC_{50} flow cytometry	0.84	0.39	0.50
EC_{50} fluorescence	1.21	0.83	0.60

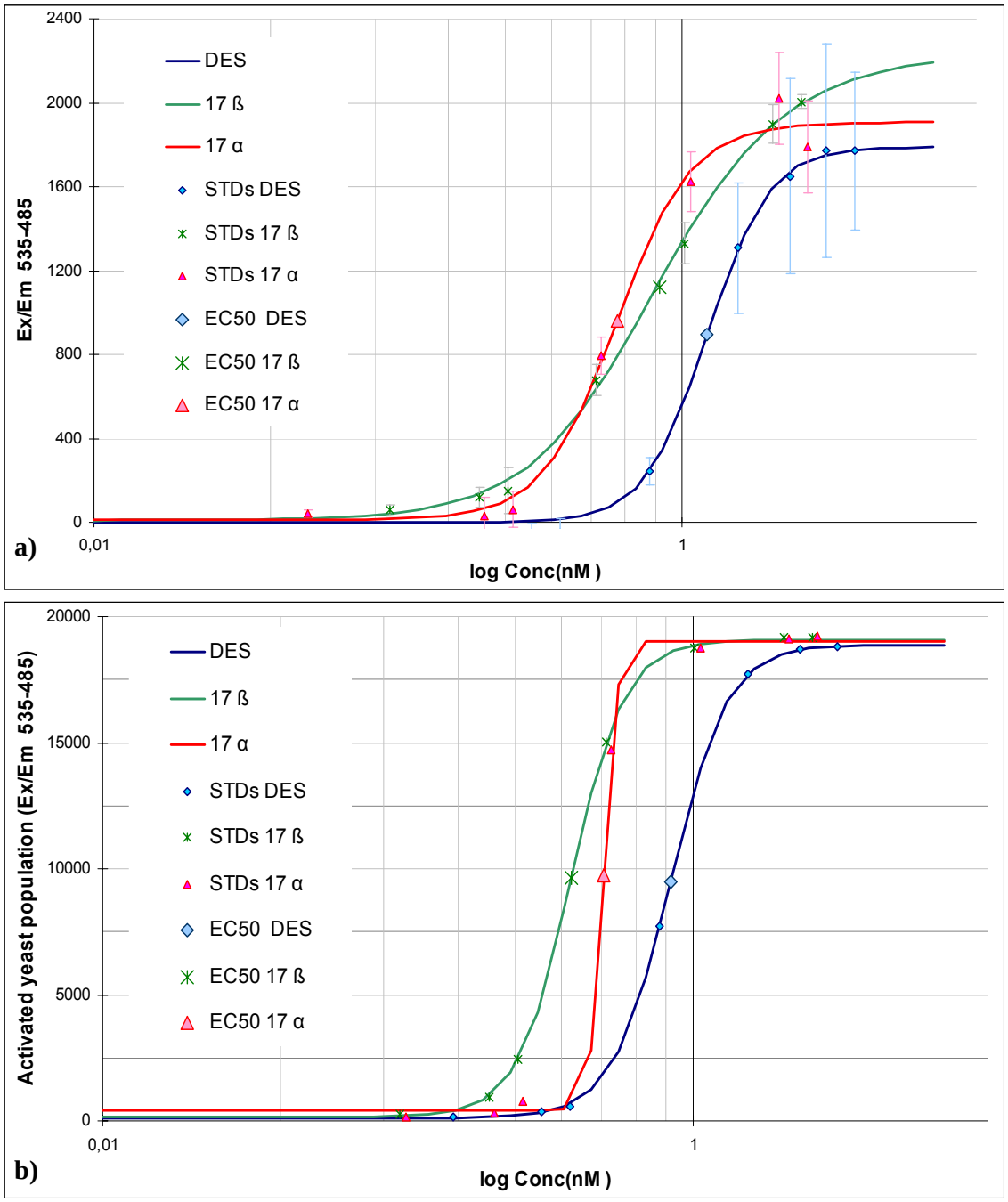


Figure 3-44: Dose-response curves after 18 hours incubation for DES, 17ß-E and 17α-E (nM). a) Fluorescence per well (n=6). b) Activated cells P2 in the total cell population.

3.7.4 AhR inducers and endocrine disrupters in forested mountain areas

The general trend is higher responses in bioassays performed with humus soils than with mineral soils. Table 3-20 gives the mean response values for the EROD bioassay (n=3) and the yeast based estrogenic bioassay (n=3).

Table 3-20: EROD results after 72 hours incubation (pg TCDD EQ/ g dry soil) and estrogenic activity (pg 17ß-Estradiol/ g dry soil) in humus (HUM) and mineral (MSL) soils (n=3).

Sampling Code	*EROD TE-value (pg TCDD/g dry soil)*	*SD*	*Estrogenicity (pg 17ß-E EQ/g dry soil)*	*SD*
HUM-CH-01-2	9.6	0.9	0.45	0.05
HUM-CH-01-4	5.1	1.2	0.24	0.01
HUM-CH-01-6	9.5	0.5	0.39	0.10
HUM-SL-33-0	6.8	1.5	0.61	0.24
HUM-SL-34-1	3.1	0.8	0.34	0.01
HUM-SL-34-2	5.3	1.9	0.36	0.03
HUM-SL-34-3	3.7	0.9	0.30	0.01
HUM-SL-34-4	5.2	0.9	0.25	0.01
HUM-IT-04-1	2.7	0.8	0.26	0.03
HUM-IT-04-3	5.9	0.5	0.28	0.07
HUM-IT-04-4	2.6	0.2	0.19	0.07
HUM-AT-47-1	2.7	0.5	0.59	0.14
HUM-AT-47-2	4.0	0.2	0.54	0.13
HUM-AT-47-3	2.4	0.6	0.44	0.13
HUM-AT-47-5	14.5	4.2	0.47	0.13
MSL-AT-47-1	< 1	-	n.d.	-
MSL-AT-47-3	3.0	0.9	n.d.	-
MSL-AT-47-5	3.2	1,1	n.d.	-
HUM-AT-57-1	3.8	1.1	0.42	0.06
HUM-AT-57-2	2.1	0.6	0.37	0.04
HUM-AT-57-3	3.0	0.6	0.35	0.04
HUM-AT-57-4	4.1	1.1	0.31	0.03
HUM-AT-57-5	6.1	0.2	0.37	0.02
MSL-AT-57-2	< 1	-	n.d.	-
MSL-AT-57-4	6.3	1.2	0.18	0.01
MSL-AT-57-5	4.6	1.1	0.18	0.04
HUM-DE-21-1	6.9	1.8	0.43	0.07
HUM-DE-21-2	17.7	0.1	0.42	0.01
HUM-DE-21-3	9.8	2.6	0.34	0.03
HUM-DE-21-4	11.7	3.4	0.37	0.07
HUM-DE-21-5	14.4	4.3	0.42	0.07
HUM-DE-23-1	5.0	0.5	0.44	0.17
HUM-DE-23-2	6.5	0.6	0.45	0.07
HUM-DE- 23-3	4.5	1.8	0.44	0.12
HUM-DE-23-4	8.5	3.0	0.39	0.01
MSL-DE-23-3	13.4	4.1	0.09	0.02

This effect was also observed in the analytical determinations, where humus soil concentrations were higher than mineral soil concentrations. There is no correlation between both bioassays but the samples that were below the limit of quantification in the EROD bioassay were also the same samples difficult to detect the estrogenic response in the yeast-

based assay. There is no trend in concentrations at the altitude profiles for estrogenic response results. The tendency to higher EROD values at the highest points of the altitude profiles is not observed for the estrogenicity values. EROD and estrogenic yeast based studies performed by Xiao *et al*. (2006) in soils from an industrialised area gave a mean of 2.5 ± 2.6 pg 17ß-E EQ g^{-1} dry soil and 10.4 ± 7.6 pg TCDD g^{-1} dry soil, meanwhile the mean values obtained in the current study are 0.37 ± 0.12 pg 17ß-E EQ g^{-1} dry soil and 6.1 ± 4.2 pg TCDD g^{-1} dry soil. Lower values in the remote areas were expected, but it is clear the higher relative amounts of AhR inducers than ER disrupters, indicating the higher abundance of persistent dioxin-like compounds than endocrine inducers in the studied forested areas.

4. CONCLUSIONS

4.1 Sampling techniques to detect and monitor POP

This study revealed the suitability of semi permeable membrane devices and coated stir bars as passive air samplers in mountainous regions for qualitative and semi-quantitative determinations of persistent organic pollutants. It is also shown that the results obtained by the passive sampling methods depend on several parameters, such as the physicochemical properties of the compounds of interest, time of exposure, temperature and variations of the temperature (seasonal periods) at which the sampling is performed.

SPMD

Lower chlorinated compounds, ΣTetraCDD/Fs and ΣPentaCDD/Fs, presented a good uptake in SPMD devices, whereas higher chlorinated homologues, OCDF in particular, were hardly detectable or not present in SPMD after 6 month exposure. SPMD accumulate lower chlorinated PCDD/Fs, in particular TetraCDD/Fs, due to their ability to uptake compounds with high K_{oa} values from the gaseous phase.

SPMD deployed consecutively for ½ year, showed a seasonal air variation for PAH compounds, with higher PAH uptake in winter than in summer. The suitability of the ½ year deployment as a direct correlation with air concentrations needs supplementary studies with PRC but the uptake of PAH in SPMD and active samplers after ½ year exposure correlated linearly. However, SPMD exposed for 1 year exhibited a decrease of low molecular PAH concentration, in comparison to the 1/2 year exposure. This point out the deviation from the linear kinetic uptake at longer exposure periods mainly for vapour phase related PAH. Therefore, for the analyzed PAH, in particular for PAH in the gaseous phase, SPMD deployments longer than ½ year are not advisable to calculate time weighted average concentrations. The differences in gas-particle distribution in the two different periods studied seem to have influenced the uptake of compounds in SPMD due to changes in the dry deposition process and presence of the PAH in the gaseous phase together with the temperature differences. A PAH decrease in SPMD was observed in altitude profiles, and no PAH could be detected at the highest elevated sites, independently of the season and the deployment time. This is related to the lower concentration of this type of pollutants far from

the sources of origin. SPMD was a very feasible tool to accumulate low molecular weight PAH but high molecular weight PAH (particle bounded) were also detected.

In this study, OCP were divided in 3 groups according to their uptake stage in passive samplers. The least lipophilic compounds ($K_{oa} \leq 8$ at 5° C) with higher volatility were no longer in the linear phase even before the 1/2 year deployment was over (Group 2: PeCB and HCB). The most lipophilic compounds were still found in the linear uptake stage, even after 1 year deployment (Group 3: the rest of the analysed OCP with $K_{oa} > 8$ at 5° C and low volatility). The Group 1 (α- and γ-HCH isomers) formed an intermediate group between the other two groups, where the uptake stage was more influenced by the geographical characteristics (temperature altitudinal gradient) due to the intermediate properties of this group. To exemplify this, α- and γ- HCH isomers in SPMD deployed for 1 year at higher altitude sites (above 2000 m a. s. l.) tend to be still in the linear uptake stage, while SPMD deployed below this altitude height did not. In contrast, compounds that belong to Group 3 were still in the linearly uptaking and Group 2 was close to or in steady state independently of the site's altitude profile. SPMD deployed 1 year at higher altitude sites tend to act as kinetic samplers. As a general tendency, an increase of concentration above 1400 m a. s. l. was observed in all compounds independently of their kinetic behaviour. In particular, pesticides tend to accumulate at the highest locations of the altitude profiles indicating the long-range origin of these contaminants.
In summary, SPMD were in different kinetic stages regarding OCP, the SPMD being very differently influenced by the temperature (thus season of exposure and altitude height of the sampling site) and inherent properties.

Coated stir bars

PAH, OCP as well as PCB were all accumulated by CSB and the change in the concentrations after two different exposure times were closely related to the compound properties in particular K_{oa} and their presence in the gaseous phase. There was no good correlation between air concentrations determined by active sampling and coated bar accumulation for OCP or PAH, but for the PCB congeners. CSB and SPMD exhibited a similar performance for the OCP and PCB families but a very different one for PAH. This confirms that these two passive samplers were acting differently regarding the mass transfer, which determined the differences in uptake behaviour. After 1 ½ year deployment, OCP with K_{oa} values higher than 8 were still in the linear uptake so a semi quantitative determination of these compounds

would be possible in CSB and SPMD. This could not be applied to the rest of the OCP family, where deployment times lower than ½ year would be necessary for quantitative or semi-quantitative determinations. Indicator and mono-ortho PCB presented more accumulation capability in CSB than in SPMD. Thus, CSB seem to be a promising tool to quantify PCB after ½ year exposure and for high chlorinated PCB even after 1 ½ year exposure. Regarding the non-ortho PCB # 126 and # 169, longer exposure deployments are required in CSB to detect adequately these compounds.

Low molecular weight PAH were not in the linear stage uptake after 1 ½ year exposure in CSB and as a consequence, a direct extrapolation of air concentrations from the uptake in the passive sampler was not possible. Shorter deployment periods (1/2 year exposure) are advisable for PAH determination in these devices.

Natural matrices: needles and soil

½ year old needles collected compounds from all the studied PCDD/F homologue groups and PCDD/F concentrations determined by active air campaigns and ½ year old needles correlated well. PCDD/F showed similar distributions at all sampling sites with a shift to lower chlorinated homologues in aged needles. In the case of PAH, needles exposed longer times accumulate compounds without significant changes in their distribution patterns.

Needles were less "selective" than SPMD, nonetheless they were able to uptake PCDD/F, PAH and OCP compounds from both the particle bound and gaseous phase. The heterogeneity of the matrix leaf did not allow determining tendencies regarding altitudinal profiles, but the needles gave a qualitative estimation of the existent compounds and tendency patterns with the time at the local sites.

The matrices humus and soil were the only matrices where all the studied compounds were found. A tendency to higher loadings at the highest altitudinal points was determined in the forest soils generating an accumulation of persistent organic pollutants in these remote areas.

4.2 Bioassays

The matrices SPMD and needles accumulated estrogenic compounds below or close to the limit of quantification, whereas humus and mineral soil loaded the highest estrogenic concentrations. Persistent compounds able to induce the AhR receptor were well detected in soil and in aged needles but not in SPMD. In sum, the best matrices to detect AhR inducers and ER inducers in the studied areas were humus and mineral soil and therefore, these are the recommended matrices to perform these bioassays in remote areas.

The new yeast-based estrogenic bioassay was implemented successfully. The estrogenic values were lower than the EROD values in relation to soils from inhabited regions, and thus, mountain forested areas accumulated in comparison more AhR inducers than ER disrupters.

In remote areas, the EROD bioassay gave a proper estimation of the overall CYP1A1 induction potential of the sample. The comparison between TEQ of bio-analytical and analytical results allows identifying additional contamination sources in the soil. As a trend, these values showed to be higher than the analytical determinations but in the same order of magnitude. This indicates the existence of lower concentrations of other AhR inducers in these remote areas not determined by the analytical determinations. In more loaded soils, the EROD activity decreased at high concentrations of non-ortho PCB or other antagonistic modulators. In conclusion, lower TEQ-WHO values in the analytical determinations than TE values in the EROD bioassay indicated contaminant sources acting as AhR agonists in the studied forested areas.

5. FUTURE RESEARCH OPTIONS

Regarding the passive sampler techniques, a better understanding of the uptake of persistent organic compounds in these devices, for example by using performance reference compounds, will be necessary to achieve more precise quantification. In this way, the air concentrations could be related to the accumulated amounts in SPMD, accomplishing complete independency of active air sampling. PRC would also allow the optimization of the deployment periods for CSB and SPMD. The calculation of sampling rates under different *in situ* conditions led to an understanding of climatological effects, due to the temperature variations. Consequently, further studies with PRC should be executed to allow a posterior quantification of POP in air by the passive techniques coated sir bar and SPMD.

Regarding SPMD, the following points could be addressed in the future to better comprehend the mechanisms of importance during the compound uptake:
The strong selectivity of SPMD for the gas phase might open new opportunities to distinguish between gas phase and aerosol by a combination of SMPD and active air sampling. Some facts, such as the good uptake of high molecular weight PAH and the poor uptake of higher chlorinated PCDD/F, all of them particle bound compounds, are interesting to address and to understand.
Unusual findings, such as the increase of more than 25-50 % of the total weight in SPMD after aerial deployment are worth to be investigated, in particular the climatological conditions that favour this extra uptake and the possible influences on the accumulation of compounds into the device.
The possible indirect photodegradation of performance reference compounds in triolein, as well as possible secondary products, would be important to understand in order to assure a correct calculation of SPMD rates, and therefore, accurate determination of the concentrations in air of POP.

Regarding bioassays, flow cytometry can be seen as a future possibility to perform yeast-based estrogenic measurements. EROD responses in relationship to the analytical determinations deserve further research in order to determine persistent compounds that at different concentrations (environmental levels) give agonistic or slightly antagonistic responses.

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Appendix I

According to Mackay (1979) the thermodynamic partitioning expression called fugacity *f* in a certain compartment is defined by:

$$f_i = \frac{C_i}{Z_i MW_i} \qquad \text{Equation 1}$$

where C_i is the concentration of the compound (g m^{-3}), MW_i the molecular weight (g mol^{-1}), Z_i is the fugacity capacity of the compartment for the compound (mol m^{-3} Pa^{-1}) and f_i the fugacity (Pa) of the compound *i*. Using the following equivalence;

$$\frac{C_i}{MW_i} = \frac{n_i}{V_i} \qquad \text{Equation 2}$$

where n_i is the amount of chemical *i* (mol) in the compartment and V_i is the medium volume (m^3) and substituting in Equation 1 leads to:

$$f_i = \frac{n_i}{Z_i V_i} \qquad \text{Equation 3}$$

For the aerial compartment, assuming gas ideal behaviour for the gaseous compound, Z_{air} is defined as:

$$Z_{air} = \frac{1}{RT} \qquad \text{Equation 4}$$

where R is the gas constant (8.314 m^3 Pa $mol^{-1}K^{-1}$), and T is the air temperature (K).
In this way, substituting the Equation 4 in the Equation 1, the fugacity in the air, f_{air} for a certain compound *i* is determined as:

$$f_{air} = \frac{C_{air} RT}{MW_i} \qquad \text{Equation 5}$$

where C_{air} is the compound concentration in the gaseous phase (g m^{-3}).
At equilibrium equal fugacity *f* exists between two phases and using the Equation 1 for the air and SPMD compartments leads to:

$$f_{air} = f_{SPMD} \Rightarrow \frac{C_{air}}{Z_{air} MW_i} = \frac{C_{SPMD}}{Z_{SPMD} MW_i} \Rightarrow \frac{C_{air}}{Z_{air}} = \frac{C_{SPMD}}{Z_{SPMD}} \qquad \text{Equation 6}$$

As the SPMD-air partition coefficient K_{sa} under steady state is given by:

$$K_{sa} = \frac{C_{SPMD}}{C_{air}} \quad \text{Equilibrium partitioning} \qquad \text{Equation 7}$$

Regrouping Equation 6 and equalling to Equation 7 is obtained:

$$K_{sa} = \frac{C_{SPMD}}{C_{air}} = \frac{Z_{SPMD}}{Z_{air}} \qquad \text{Equation 8}$$

Under steady state, a partition coefficient is thus, the ratio of two Z values. The Z values, which are both functions of the chemical and the medium, can be viewed as a chemical's solubility in, or affinity to a phase (Mackay, 1979). Therefore, high concentrations are reached in phases for which Z is large. Considering depreciable the membrane fugacity capacity ($Z_{membrane} << Z_{triolein}$), Z_{SPMD} can be approximated as:

$$Z_{SPMD} = Z_{membrane} + Z_{triolein} \approx Z_{triolein} \qquad \text{Equation 9}$$

Considering the SPMD such as an "aerial fat compartment" able to accumulate compounds and bearing in mind that K_{sa} can be approximated by K_{oa}, $Z_{triolein}$ can be defined as:

$$Z_{triolein} = \frac{(1-\Phi)K_{sa}}{K_H} \approx \frac{(1-\Phi)K_{oa}}{K_H} \qquad \text{Equation 10}$$

K_H is the Henry's law constant (Pa m^3 mol^{-1}) and Φ is the particulate-phase fraction of the compound (thus, 1-Φ is the gas-phase fraction). According to Harner *et al*. (2000), Φ can be estimated using the equation:

$$\Phi = \frac{K_p TSP}{(K_p TSP + 1)}$$ Equation 11

where K_p is the particle-gas partition coefficient ($m^3\ \mu g^{-1}$) and TSP is the total suspended particulate concentration ($\mu g\ m^{-3}$). Note that Φ is dimensionless per definition.

Substituting the Equation 10 in the Equation 1 the fugacity of a compound in the SPMD compartment can be calculated as:

$$f_{SPMD} = \frac{C_{SPMD} K_H}{(1-\Phi) K_{oa} MW}$$ Equation 12

The Henry's law constant is temperature dependent and is usually given at 25 °C. The re-calculation at the working temperature follows the Clausius-Clayperon formula. The temperature correction is not applied in the current work since the error from calculation approximations is higher than the error of the Henry's law constant at 25 °C. On the other hand, the K_{oa} values need to be corrected at the average temperature. For the calculations, a temperature of 5°C (278 K) for K_{oa} values was considered.

The fugacities in the air and SPMD compartments were calculated with the equations 5 and 12, respectively (see Section 3.6.3) considering the fraction of the compound in the particulate phase depreciable (Φ≈0). As an internal calculation check, the equation 8 was used.

Appendix II

Scanning electron microscopies of SPMD deployed at a) DE-23-1 (830 m a.s.l.) and b) DE-23-4 1420 (m a.s.l.) for 1 year. Courtesy of the IFEM: Institut für Raster-Elektronenmikroskopie "Dr. Rudolf Hünert" (Hamburg, Germany). Scale 1mm.