EXPLORING THE UTILITY OF CHIRAL SIGNATURES TO FURTHER UNDERSTANDING OF SOIL-TO-HERBAGE TRANSFER OF PERSISTENT ORGANIC POLLUTANTS (POPs).

By

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MChem (Hons)

A thesis submitted to the University of Birmingham for the degree of

Doctor of Philosophy (Ph.D)

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September 2011

Abstract

Chiral signatures were utilised as a means of determining the pathways of PCBs in the environment. Concentrations of HBCD diastereoisomers, enantiomers and degradation products were also determined in top soils from both the UK and Australia.

Concentrations of PCBs 28/31, 52, 101, 118, 138, 153, and 180 and enantiomer fractions (EFs) of PCB 95 and 136 were determined in air, top soil and grass from an urban site in Birmingham, UK. Samples were taken approximately every 14 days at 5 graduating heights from the ground in summer 2009 (114 days) and spring 2010 (84 days). EFs of PCB 95 in air at 3 cm height (average of 0.453 in 2009 and 0.468 in 2010), differed significantly ($p<0.05$) from the racemic EFs in air at 10, 40, 90, and 130 cm. The EFs of PCB 95 in soil were on average 0.452 in 2009 and 0.447 in 2010 and matched those in air at 3 cm particularly in the 2009 campaign. Grass displayed an average EF of 0.468 (2009) and 0.484 (2010); values which were intermediate between those in soil and the racemic EFs in air measured at 10 cm and above. These data imply that at the study site, PCBs volatilise from soil to an extent discernible only at the soil:air interface, and that PCBs in grass arise due to foliar uptake of volatile emissions from soil. Atmospheric concentrations of ΣPCBs increased significantly ($p<0.05$) with increasing height above the soil surface. This combined with the PCB 95 chiral signature data, suggests that the influence of PCB emissions from soil on airborne concentrations decreases with height while emissions from indoor air increases.

HBCD chiral signatures were found to be racemic or near-racemic in all the air, grass and soil samples from the same urban site in Birmingham, UK used for sampling PCBs. Soils from 24

sites across the UK were also found to contain near racemic chiral signatures of HBCDs. This indicates that enantioselective microbial degradation is not occurring and the sites were unsuitable for a study like that used for PCBs to determine pathways of HBCDs into plants using chiral signatures.

Concentrations of ∑HBCD from soils from the UK (n=24) were found to be 22 ng/g ranging between <0.03 to 420 ng/g. By comparison, the average concentration of ∑HBCD in soils from Australia (n=17) was 0.74 ng/g ranging between <0.0005 to 5.6 ng/g. Degradation products of HBCD, pentabromocyclododecenes (PBCDs) and tetrabromocyclododecadienes (TBCDs) were also semi-quantitatively determined in the soil samples. In the UK soils, PBCDs and TBCDs were determined in 7 and 6 of the soil samples respectively with concentrations ranging from 10-7300 pg/g for ∑PBCDs and 10-1300 pg/g for ∑TBCDs. In the Australian soils only TBCD was detected in soil at concentrations ranging from 2.3 to 450 pg/g ∑TBCDs. A preliminary environmental budget found soil to be the principal sink for HBCD in the UK.

Acknowledgments

My greatest thanks go to my supervisor Professor Stuart Harrad for his invaluable knowledge, advice, and encouragement. I greatly appreciate the time and help received throughout the past four years.

I gratefully acknowledge funding from the Natural Environment Research Council and the Foods Standards Agency.

My thanks to everyone I have met and worked with in room 216 for many happy memories. Particular thanks go to Dr. Mohamed Abdallah for always taking the time to answer my many questions and for giving me help and advice throughout my entire Ph.D.

Finally my thanks go to my family for their continued support and encouragement.

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ABBREVIATIONS

BFR	Brominated Flame Retardant
BSEF	Bromine Science and Environmental Forum
CBs	Chlorobenzenes
DEFRA	Department for Environment, Food and Rural Affairs
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
DW	Dry Weight
ECHA	European Chemical Agency
ee	Enantiomeric excess
EF	Enantiomeric fraction
EI	Electron impact
EPA	Environmental Protection Agency
EROS	Elms Road Observatory Site
ER	Enantiomeric Ratio
FSA	Food standards agency
GC	Gas chromatography
GIT	Gastrointestinal Tract
HBCD	Hexabromocyclododecane
HEPT	Heptachlor
HEPX	Heptachlor epoxide
IS	Internal standard
K_{OW}	Octanol-water partition coefficient
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
OC	Organochlorine Pesticides
ONS	Office for National Statistics
PAH	Polycyclic aromatic hydrocarbon
PAS	Passive air sampler

PBCD	Pentabromocyclododododecene
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
POP	Persistent organic pollutant
PME	Public microenvironment
QA/QC	Quality assurance/quality control
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RRF	Relative response factor
RSD	Relative standard deviation
SES	Sampling evaluation standard
S/N	Signal to noise
SRM	Standard reference material
TBCD	Tetrabromocyclododecadiene
TBCDe	Tetrabromocyclododecene
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalent
TPEM	Two-photon excitation microscopy
TSM	Total suspended matter
UK	United Kingdom
WHO	World Health Organisation
WT	Wet weight
XPS	Extruded polystyrene

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CHAPTER 1: INTRODUCTION

1.1 Synopsis

Persistent organic pollutants (POPs) are of interest due to their potentially dangerous effects on human health and the environment. Methods of determining the sources of POPs and how they are transferred in the environment are important areas of interest. Of particular interest, is the elucidation of mechanisms via which POPs enter and transfer through the food chain. Such studies are motivated by the desire to minimise human exposure to POPs via food. There has been much recent research which has utilised chiral signatures as a means of determining pathways in the environment (Lehmler et al., 2010, Robson et al., 2004; Warner et al., 2005; Wong et al., 2004).

Two groups of POPs which are of interest are polychlorinated biphenyls (PCBs) and hexabromocyclododecane (HBCDs). PCB production and new use was banned in the 1970s but existing stocks are still in use and they continue to pose a problem due to their environmental persistence (Li et al., 2010). HBCD is a high production brominated flame retardant (BFR), which is being found at increasing levels in the environment (Law et al., 2005). Some PCBs and HBCDs have chiral properties. The commercial product of HBCD contains predominantly three diastereoisomers α, β, and γ, each of which exist as enantiomer pairs.

The chirality of POPs can be used to gain further understanding into their environmental behaviour. Recent studies have utilised the chiral properties of organochlorine chemicals such as α-HCH, heptachlor, chlordane, and PCBs to distinguish between sources of such contaminants to a given environmental compartment (Bidleman et al., 1998, Bidleman and

Falconer, 1999, Robson and Harrad, 2004, Warner et al., 2005). For POPs that display chirality, the relative abundances of the two enantiomers present (i.e. the chiral signature) in the commercial formulations are equal (i.e. racemic). Enantiomers have the same chemical and physical properties and will transport in the environment in the same way, e.g. by volatilisation, but they may react differently to biological processes (Bidleman and Falconer, 1999). Therefore deviation from the racemic state, suggests there has been a biologically-mediated change.

POPs can potentially enter the food chain either from the atmosphere or from soil by a number of different mechanisms. Air to plant transfer is believed to be the major route for organic chemicals entering plant foliage (Collins et al., 2006). However, it has been suggested recently that soil contamination may exert a greater influence on the concentrations of PCBs in grass than previously believed. A study by Harrad et al., compared chiral signatures of PCBs 95 in air sampled 1.5 m from the surface, soil and grass (Harrad et al., 2006). Interestingly, the results showed that while the chiral signatures in "bulk" air were racemic; those seen in soils and grass were in many cases similar, particularly in the warmer sampling periods. The implication of this study is that PCBs in grass could potentially be arising from volatilisation from soils. A limitation of this paper was that the samples were procured as part of several different studies, and while taken at the same location, were not taken over the same time periods. However, the hypothesis generated is interesting and an important area that needs further investigation. It also poses the question as to whether this is also the case for other physicochemically similar classes of contaminants, e.g. HBCDs. Furthermore, the chiral properties of some HBCD diastereoisomers means that the approaches employed to investigate PCBs may be applied.

1.2 Mechanism of uptake of POPs

1.2.1 Introduction

The uptake of POPs by vegetation is believed to be a major route by which humans will become exposed; this could be by eating the vegetation directly or from eating meat, eggs or dairy products from animals which have eaten contaminated vegetation. The mechanism of uptake of POPs by plants is not fully understood. The two principal environmental pathways via which POPs can enter the food chain is either from the atmosphere, which can enter the plant via foliage, or from the soil. From these two sources there are number of mechanisms by which the POPs can then enter the plant. Figure 1.1 illustrates the routes via which POPs can potentially enter plants.

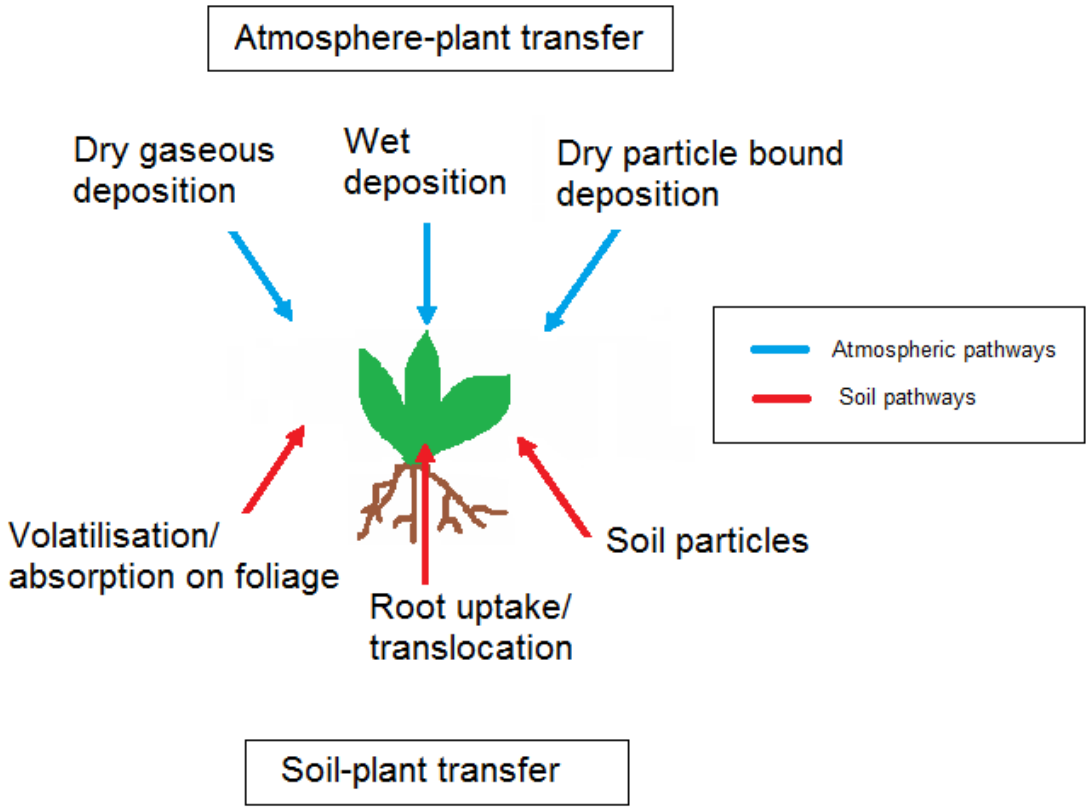


Figure 1.1: Pathways of POPs into plants (McLachlan 1996)

There are many different conditions and properties that will affect how POPs are transferred between air, soil and plants. These include the physicochemical properties of the POP, the properties of the soil, the species of plant and environmental conditions such as climate.

The physicochemical properties of the POPs are important in determining how they will be transferred in the environment. Table 1.1 shows a number of important physicochemical properties that govern the environmental fate and behaviour of POPs.

Table 1.1 Physicochemical properties affecting environmental behaviour of POPs

Parameter Abbreviation	Parameter	Environmental relevance
$\mathbf{K_{OW}}$	Octanol-water partition coefficient	Ability of a chemical to partition between water and lipids
$\mathbf{K_{OA}}$	Octanol-air partition coefficient	Ability of a chemical to partition from air to lipids e.g. between vapour and particle phase
$\mathbf{K_{OC}}$	Organic carbon water partition coefficient	Ability of a chemical to partition between organic matter and water
$\mathbf{H_C}$	Henry's constant	Tendency for a chemical to partition from aqueous solution to air
$\mathbf{V_P}$	Vapour pressure	The ability of a chemical to exist as a vapour
$\mathbf{W_S}$	Water Solubility	The solubility of a chemical in water

(From Evans, 2007)

The properties of the plant itself are also an important influence on the mechanism by which POPs will transfer and to what extent. The structure of a leaf is shown in figure 1.2. The major pathway via which gaseous, lipophilic POPs enter plant foliage is through the cuticle, a lipid surface covering the leaves of plants, the structure of which varies between plant species (Barber et al., 2004). It has a waxy surface which stores lipophilic compounds and repels

more water soluble compounds (Duarte-Davidson and Jones, 1996). Another route via which POPs can enter the plant is the stomata which are tiny pores typically found on the underside of leaves. Although the major route of uptake has been believed to occur via the cuticle, the stomatal pathway is also an important route. This is particularly true when the permeability of the cuticle is low, as it was found that the uptake rate for PCBs was higher when the stomata were open (illuminated) than when the stomata are closed for a plant with high stomatal density (Barber et al., 2002a). Interspecies variation in plant uptake is also dependent on the properties of the POP, as indicated by the fact that there is greater variability for more volatile compounds including PCBs (Böhme et al., 1999).

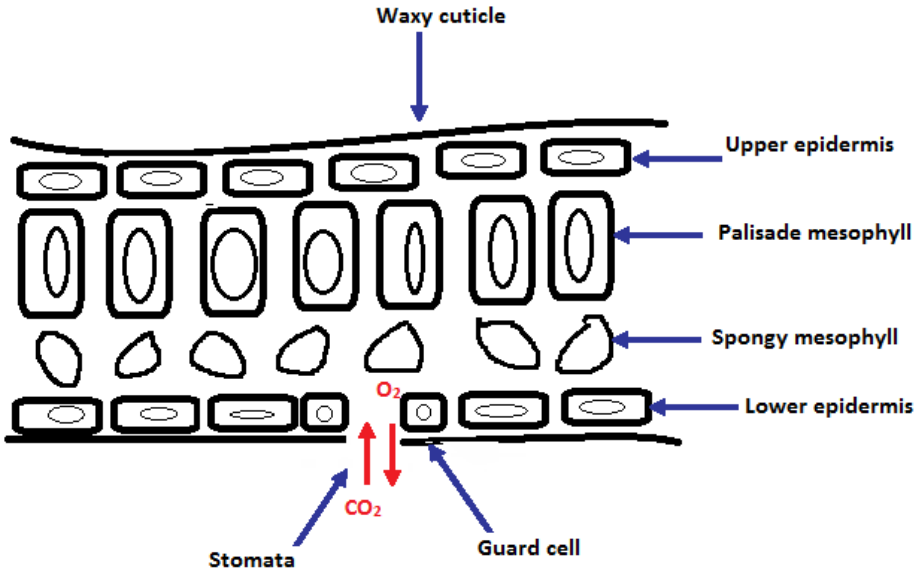


Figure 1.2: Structure of a leaf showing the two main routes of POPs into the plant, the waxy cuticle and the stomata.

1.2.2 Soil to plant transfer

Soils are a significant reservoir for POPs in the environment (Dalla Valle et al., 2005). For soil to plant transfer the POPs can either enter via the root of the plant, by adherence of soil

particles to foliage, or through volatilisation from soil, with subsequent uptake by foliage (McLachlan, 1996). It is believed that such pathways via which POPs enter plants from soil are negligible, except when the soil is highly contaminated, indicating that this is not the major pathway via which POPs enter plants (Barber et al., 2004). However the soil to air to plant pathway has been found to be significant in a number in studies, particularly in areas of high soil contamination (Barber et al., 2004; Trapp and Matthies, 1997).

1.2.2.1 Uptake via Roots

POPs have been detected in plant roots in a number of studies, including PCBs found in carrots (Currado and Harrad, 1999a), PCBs and organochlorine (OC) pesticides in radishes (Mikes et al., 2009) and PAH in root crops (Dennis et al., 1983). In the soil to root mechanism, compounds diffuse passively into the root and sorb onto the lipids contained within. There is equilibrium between the soil solid and soil pore water and the pore water and root lipid. More lipophilic compounds have a greater tendency to partition into the plant root than hydrophilic compounds (Collins et al., 2006). The structure of the plant is an important factor in this process. Many POPs are lipophilic and so plants which contain higher levels of lipids can uptake and store potentially higher levels of POPs (Collins et al., 2006). The type of root possessed by the plant also influences uptake; for example in plants with fine roots, diffusion between the soil and root is higher and attainment of equilibrium is more facile compared to plants with thicker roots (Trapp and Matthies, 1995).

For the levels of POPs in the plant foliage to be affected by this mechanism, translocation of the POPs by the xylem of the plant must occur after the uptake by the root. The majority of lipophilic POPs including PCDD/F, organochlorine pesticides, PCBs and PAHs partition into the epidermis of the root but not enter the inner root or xylem of the plant (Simonich and

Hites, 1995). Many studies have concluded that translocation of POPs from the root is negligible, including PCDD/F into grass (Welsch-Pausch et al., 1995) and Chlorobenzenes (CBs) (Wang and Jones, 1994). Plants grown in a medium contaminated with anthracene and phenanthrene were observed using two-photon excitation microscopy (TPEM) and found that the chemicals did not reach the phloem/xylem of the plant and that translocation from root to foliage was not a significant pathway (Wild et al., 2005). However, Zhang et al., have found that plants from the Cucurbita genus (i.e. courgettes, pumpkins and squashes) exhibit greater translocation in the case of dioxins (Zhang et al., 2009). This was also seen in earlier work by Hülster et al., in the case of courgettes (Hülster et al., 1994). Within this genus there is found to be variation in uptake of POPs among different subspecies of plant (Inui et al., 2008).

1.2.2.2 Soil particles to foliage

When soil particles are suspended in the air they can become deposited on the leaves of plants. POPs that are present in the soil particles can then be absorbed onto the cuticle of the plant leaf via dry or wet deposition (Collins et al., 2006). Soil particles to vegetation is the major route for PCDD/Fs entering plants from soils, dependent on a number of factors including plant species, the properties of the soil and the climate (McLachlan, 1996).

1.2.2.3 Volatilisation from soil

The final way in which POPs in soil can enter a plant is through volatilisation. POPs can volatilise from contaminated soil and undergo uptake by the plant leaves, rather than uptake by the root. Volatilisation is affected by temperature as vapour pressure increases with temperature. It has been observed in numerous studies that there are seasonal variations in the atmospheric concentrations of POPs (Halsall et al., 1995, Wania et al., 1998, Currado, and

Harrad 2000; Harrad and Mao, 2004; Bozlaker et al., 2008). This may be related to greater volatilisation in warmer summer months.

A study on allotment gardens saw a decrease in atmospheric concentrations with increasing height above the soil surface indicating that PCBs were volatilising from the soil (Krauss et al., 2004). Similarly, Finizio et al., found there to be a decrease in concentration of OC pesticides with increasing height ranging from 5-140 cm from the surface suggesting that the soil (concentrations ranging between 42-2496 ng/g) was a significant source to the atmosphere (Finizio et al., 1998). This study also found there to be a deviation from racemic of chiral OCs α-HCH, Heptachlor (HEPT), Heptachlor epoxide (HEPX) and o,p'-DDT in air samples which was consistent with that seen in soils. In the case of α-HCH a trend was shown with height with the greatest deviation seen specifically at air measured at 5 cm from the ground which was close to that found in soil. This indicated that volatilisation from soil was contributing to the concentrations in air. Both these studies were from contaminated sites which may be why volatilisation was seen to exhibit such an influence on the surrounding air. It has been found from modelling of the soil-air-plant pathway that at high levels of contamination of between 10-100 mg/kg that the soil-air pathway is more significant than background air-plant (Collins and Finnegan, 2010). A modelling study of volatilisation of PCDD/F from soil to vegetation found that the contamination would occur at the lowest few centimetres of the plant and that it would be significant for highly polluted soils (Trapp and Matthies, 1997).

Understanding how POPs transfer between soil and the atmosphere is important in determining the environmental fate of POPs. Soil properties including moisture content and soil organic content will affect how POPs will exchange between the soil and air. Hippelein

and McLachlan developed a method for determining the soil/air partition coefficient (K_{SA}) (Hippelein, and McLachlan 1998). The K_{SA} is dependent on both temperature and humidity with K_{SA} increasing with decreasing temperature (Hippelein and McLachlan, 2000).

Volatilisation was proposed as the main reason for a decrease in concentrations of PCBs in UK soils over time (Harner et al., 1995). It was found that the loss of PCBs from artificially contaminated soil was also most likely to be due to volatilisation (Ayris et al., 1999). Volatilisation was identified as an important route of CBs from soil (Wang and Jones, 1994). Soils are a major sink for PCBs and it has been suggested that volatilisation from soil strongly influences the concentrations of PCBs in air (Harrad et al., 1994). However, given that PCB manufacture and use ceased ~three decades ago, recent studies using PCB chiral signatures have suggested that ventilation of contaminated indoor air rather than volatilisation from soil exerts a greater influence on contemporary outdoor air concentrations (Jamshidi et al., 2007).

1.2.3 Air to plant transfer

Air to plant transfer is believed to be the major route for organic chemicals entering plant foliage. POPs in air can be transferred to plants via either:

1. dry gaseous deposition,
2. wet deposition
3. particle deposition

Dry gaseous deposition is the uptake of POPs to the plant surface via the diffusion of gaseous POPs from air (McLachlan, 1996). A study of the uptake of 5 PCB congeners and 4 OC pesticides found dry gaseous deposition to be the main uptake pathway (Umlauf et al., 1994).

This was found to be the major pathway for PCDD/F into grass (Welsch-Pausch et al., 1995) Wet deposition can occur via rain, fog or dew either when the POP is dissolved in water droplets, or from particles present inside water droplets (McLachlan, 1996). Particle deposition refers to the transfer of POPs via adherence to the plant surface of particles containing POPs which are then sorbed by the plant surface.

The contribution of these three pathways to the uptake of POPs from air to plants is dependent on a number of factors including the concentration of the POP, the type of plant and environmental conditions such as temperature (McLachlan, 1999). A study which looked at the temperature dependence of the partitioning of PCBs between air and rye grass (*Lolium multiflorum*) found that plant/air partitioning was strongly dependent on temperature. (Kömp and McLachlan 1997a). As the temperature decreases it results in increased partitioning of POPs from air to plants (Barber et al., 2004). Species is an important influence on how plants take up POPs. In a study by Kömp and McLachlan the plant/air partition coefficients (K_{PA}) varied by up to a factor of 20 between 5 different species (Kömp and McLachlan 1997b). It has been found that uptake rates for PCBs in plants were greater at ambient wind speeds (2 m s^{-1}) than in still air (Barber et al., 2002b). A study by Hung et al. found that air:grass exchange of POPs is governed by two distinct categories of behaviour, a fast exchange independent of K_{OA} and slower exchange over weeks which is related to the K_{OA} (Hung et al., 2001).

1.2.3.1 The McLachlan framework

A framework developed by McLachlan can be used to identify the main uptake route to plants (McLachlan, 1999). It uses the octanol:air partition coeffient (K_{OA}) which is an expression of the ability of a chemical to partition between air and lipids. K_{OA} is dependent on temperature

(Harner and Bidleman 1996, Kömp and McLachlan 1997c.). The framework describes the uptake of POPs by plant foliage into three principal categories:

1. Equilibrium partitioning
2. Kinetically limited gaseous deposition
3. Wet and particle bound deposition

In the case of equilibrium partitioning, equilibrium is reached between the vapour phase and the surface of the leaf whereas in kinetically limited deposition the POP is continually taken up over the growing period due to a large storage capacity of the plant. This framework uses the following equation to describe the uptake of POPs by plants via equilibrium partitioning:

$C_V/C_G = mK_{OA}^n$ (Equation 1.1)

Where C_V is the concentration of the POP in the plant/pasture (mol/m^3), C_G is the atmospheric concentration of the POPs in the gas phase (mol/m^3) and m and n are constants which are dependent on factors such as the species of the plant.

For kinetically limited deposition the framework uses the equation

$C_V/C_G = Av_{GG}\, t/V$ (Equation 1.2)

Where A is the surface area of the vegetation, v_{GG} is the mass side transfer coefficient (m/h), t is the time of exposure and V is the volume of the vegetation (m^3). In this case C_V/C_G is independent of $K_{OA.}$

For wet and particle bound deposition the equation is

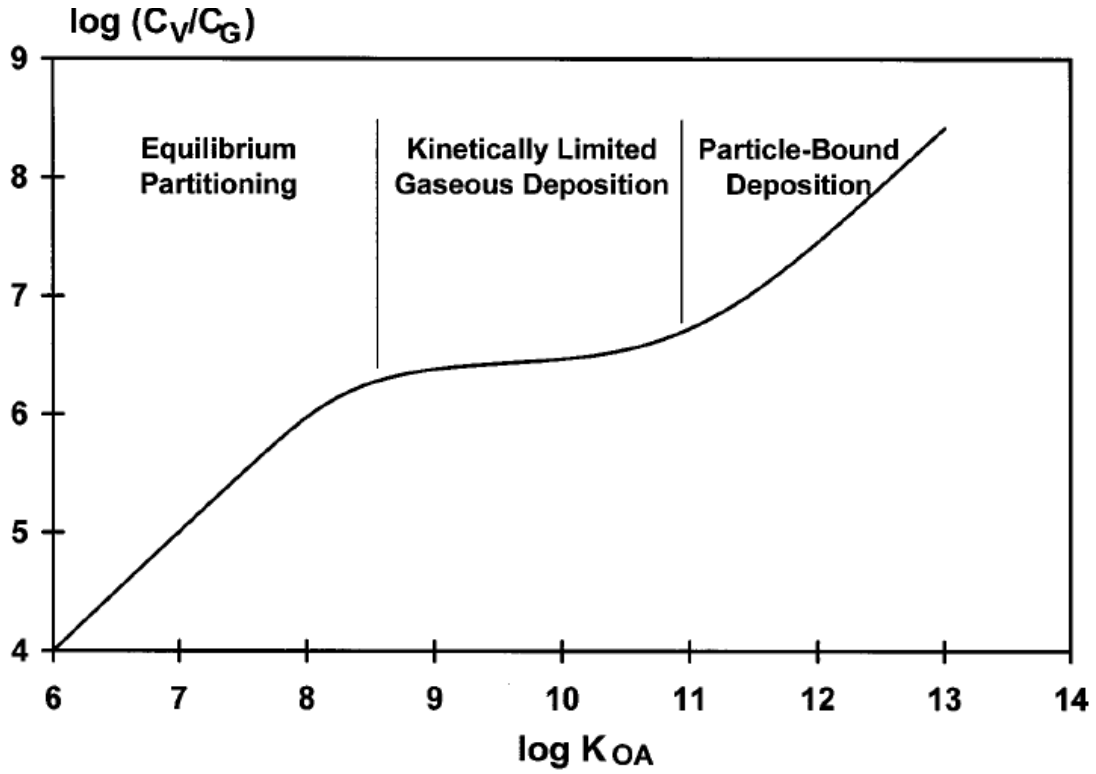
$C_V/C_G = v_P AB \times TSP \times K_{OA} / (Vk_E)$ (Equation 1.3)

Where v_P is the deposition velocity of the particle bound contaminant to the vegetation surface, B is a constant ($m^{-3}\mu g^{-1}$), TSP is the total suspended particulate matter concentration in air (μm^{-3}) and k_E is the 1st order rate constant describing erosion of the particle bound chemical from the vegetation surface (h^{-1})

A plot of log (C_V/C_G) vs Log K_{OA} can be used for identifying the primary process of plant uptake for more volatile POPs. In the case of less volatile POPs, C_G cannot always be determined as the compounds may be almost exclusively present in the particle phase. Therefore a plot of log (C_V/C_P) vs log (C_P/C_G) can be used to elucidate the principal uptake mechanism. The plots shown in figure 1.3 have three sections corresponding to the three uptake processes of equilibrium partitioning, kinetically limited gaseous deposition, and particle bound deposition. From these plots the dominant uptake process can be identified.

This plot has been used in the case of transfer of PCBs between air and grass where there was found to be a linear relationship between log (C_V/C_G) vs Log K_{OA} indicating equilibrium partitioning (Currado and Harrad, 1999b). However this study found that octanol is not always a good substitute for plant lipid, with Kömp and McLachlan reporting the uptake of POPs to be dependent on plant species (Kömp and McLachlan 1997b).

a.



b.

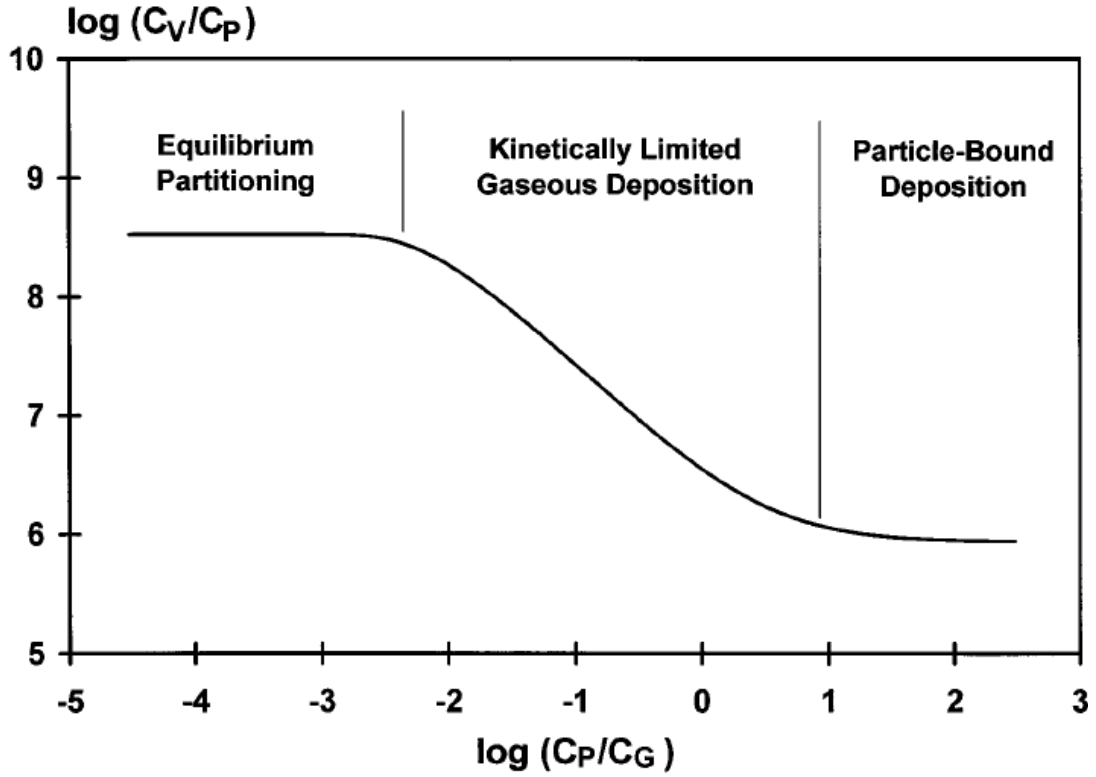


Figure 1.3: Plot of a.) log (C_V/C_G) vs Log K_{OA} for identifying the primary process of plant uptake for more volatile POPs and b.) log (C_V/C_P) vs log (C_P/C_G) for identifying the uptake of less volatile POPs (McLachlan, 1999)

1.2.4 Summary

The mechanisms via which organic pollutants enter plants are an important area of interest. It has implications for the biogeochemical cycling of POPs and determines the potential routes into the food chain and therefore human exposure.

The process of uptake of POPs from air by grass is a complex process dependent on many factors. The physicochemical properties of the POP in question, the type of the plant and climate conditions all play important roles in how POPs enter plants.

Air to foliage transfer is believed to be the major route of POPs into plants. The influence of volatilisation from soil followed by foliar uptake is not yet fully understood, although there is evidence that in particularly highly contaminated soils this route is significant. Although there have been many advances in understanding in recent years there remain gaps in knowledge about these complex processes.

1.3 Chiral signatures as an environmental forensics tool

1.3.1 Background to chirality

Isomers are compounds with the same chemical formula, but with atoms arranged differently. They can either be structural, where the atoms are bonded together in different ways or stereoisomers, which have the same atomic bond structure, but the positioning of the atoms in space differs.

Stereoisomers of a molecule, which are non-superimposable mirror images of each other are called enantiomers. Enantiomers rotate a plane of polarised light in opposite directions and so

are described as having optical activity and are called chiral. Compounds that are superimposable with their mirror image are described as optically inactive and are called achiral.

For chiral compounds each enantiomer in a pair is labelled to designate the direction it rotates polarised light. Enantiomers that rotate clockwise (dextrorotatory) are labelled (+) and those which rotate anticlockwise (levorotatory) are labelled (-). When there are equal amounts of each enantiomer it is called a racemic mixture. The relative abundance of each enantiomer in a pair is referred to generically as the chiral signature. This may be expressed mathematically in a number of ways, the most common being enantiomeric ratio (ER), enantiomeric excess (ee) and enantiomer fraction (EF). Enantiomeric ratio is expressed as:

$ER = A_+/A_-$ or $EF = A_1/A_2$ (Equation 1.4)

Where A is the peak area of the enantiomers, and A_1 and A_2 correspond to the order the enantiomers elute when the identity of (+) and (-) enantiomers is not known.

The enantiomeric excess (ee) is the excess of one enantiomer over the other (Vetter and Schurig, 1997). It is expressed by the following equation:

$$ee = \frac{A_1 - A_2}{A_1 + A_2}$$ (Equation 1.5)

where A_1 and A_2 are the amounts of the two enantiomers and A_1 is the major enantiomer. The ee will range from 0 for a racemic mixture to 1 for pure A_1 (Vetter and Schurig, 1997). It can also be expressed as a percentage where:

$$\%ee = \frac{A_1 - A_2}{A_1 + A_2} x100 = \%A_1 - \%A_2 \qquad \text{(Equation 1.6)}$$

The enantiomer fraction is expressed as:

$EF = A_+ / (A_+ + A_-)$ or $EF = A_1 / (A_1 + A_2)$ (Equation 1.7)

Where A_1 is the first eluting enantiomer and A_2 is the second eluting enantiomer. All these values are used, although Harner et al. argue that the preferred value for environmental analysis is the enantiomer fraction (Harner et al., 2000). This is because the enantiomer fraction allows for more meaningful representation of graphical data. Enantiomer fractions always fall between 0 and 1 and a racemic mixture will have an EF of 0.5.

1.3.2 Chirality and POPs

Many POPs exhibit chirality which can be used as a means of determining environmental processes (Wong, 2006). For many POPs degradation, bioaccumulation, persistence and toxicity show chiral dependence (Smith, 2009).

For those POPs that display chirality, the relative abundances of the two enantiomers present (i.e. the chiral signature) in the commercial formulations are equal (i.e. racemic). Enantiomers have the same chemical and physical properties and will transport in the environment in the

same way, e.g. by volatilisation, but they interact differently with other chiral compounds, so biodegradation and metabolism may take place at different rates for each enantiomer (Bidleman and Falconer, 1999). In soils for example, POPs will undergo microbial biodegradation, a process which may be enantiomer specific. Chiral signatures can therefore be used as a tool to determine the source and environmental fate of POPs. POPs may be identified as arising from older "legacy" sources, for example soil and water that has been contaminated in the past could be sources of continued volatilisation into the atmosphere, or they could arise from "new" sources from locations were they remain in use (Bidleman and Falconer, 1999).

Chiral signatures have been used for source appointment of PCBs in outdoor air. Appreciable enantioselective degradation of PCBs 95, 136 and 149 in top soil from 2 sites in the West Midlands was reported, alongside racemic signatures in outdoor air from the same locations (Robson and Harrad, 2004). Further work at 10 West Midlands locations compared chiral signatures of outdoor air and soils with each other and with those in indoor air, were it was found that racemic chiral signatures in outdoor air did not match with that seen in soil but matched closely to that seen indoor air (Jamshidi et al., 2007). Combined, these studies suggest strongly that PCBs in outdoor air come from indoor air and not from soil. This is in contrast to the previously held belief that PCBs in air originated primarily via volatilisation from soil (Harrad et al., 1994). Chirality was also used by Kurt-Karakus et al., who found that the enantiomer specific degradation of organochlorine pesticides (α-HCH, cis- and trans-chlordane and o,p' DDT) in soils varied to a great extent over horizontal distances of only a metre or so and vertical depths of a few centimetres (Kurt-Karakus et al., 2007). In research examining soil to root transfer, Lee et al. observed that chlordane appeared to undergo enantioselective transport from soil into the root of a zucchini plant (Lee et al., 2003).

1.4 Polychlorinated biphenyls (PCBs)

1.4.1 Introduction

Polychlorinated biphenyls (PCBs) have been used in a wide range of applications, including as dielectric fluids and as flame retardants (Ross, 2004). There are 209 congeners and they have the general formula $C_{12}H_{10-x}Cl_x$. They are hydrophobic and persistent in the environment, and are accumulated easily by aquatic organisms (Benicka et al., 1998; Buckman et al., 2006). PCB persistence is believed to increase with increasing chlorination (Harrad et al., 1994). Due to the effect of PCBs on human health and the environment their production was banned in the UK in the 1970s. (Jamshidi et al., 2007). Despite this, the environmental legacy of PCBs remaining as a result of this past use due to their environmental persistence, as well as continuing releases of those still in use continue to pose a problem for the environment.

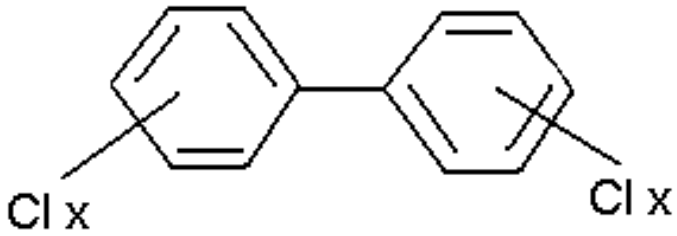


Figure 1.4: Generic Structure of PCBs

Table 1.2 Physicochemical properties of selected PCB congeners (Li et al., 2003)

Congener	Homologue group	Molecular weight	Henry's Law Constant ($Pa/m^3/mol$)	Melting point (^0C)	Solubility (g/L)	Vapour pressure (Pa)	Log K_{OW}	Log K_{OA}
PCB 28	Tri	257.5	30.20	58	8.85×10^{-4}	3.35×10^{-2}	5.66	7.85
PCB 52	Tetra	291.9	25.12	86.5	4.78×10^{-4}	1.58×10^{-2}	5.84	8.22
PCB 101	Penta	326.4	25.43	77	1.02×10^{-4}	3.33×10^{-3}	6.38	9.14
PCB 118	Penta	326.4	14.45	110	6.83×10^{-5}	9.62×10^{-4}	6.69	9.36
PCB 153	Hexa	360.9	19.95	103	3.07×10^{-5}	4.43×10^{-3}	6.87	9.44
PCB 138	Hexa	360.9	30.20	79	1.87×10^{-5}	5.04×10^{-4}	7.22	9.66
PCB 180	Hepta	395.3	30.40	112	1.32×10^{-5}	1.29×10^{-4}	7.16	10.44

1.4.2 Human exposure to PCBs and its health effects

PCBs have been found to have adverse health effects in animals and humans. Health effects associated with PCBs include carcinogenicity, particularly of the digestive system, liver and malignant melanoma; reproductive deficiencies, immunological changes and dermatological effects (WHO, 2003). Studies have indicated that PCBs induce carcinogenic effects in laboratory animals (Safe, 1989). Koopman-Esseboom et al., found that elevated dioxins and PCBs in pregnant women can alter the human thyroid hormone status (Koopman-Esseboom et al., 1994). There is evidence from a number of studies that PCBs have exhibit an effect on neurodevelopment in children due prenatal exposure (Schantz et al., 2003). PCB poisoning causes damage to the skin including chloracne, hyperpigmentation, loss of hair and porphyria (George et al., 1988).

A review into the toxic effects 35 years after a mass poisoning in northern Kyushu to PCBs and PCDFs found there to be many long term health effects (Masuda, 2005). At least 1860 individuals were poisoned by a Japanese commercial brand of PCBs in rice bran oil. Initial symptoms included acneform eruptions, dermal pigmentation and increased eye discharge. In the longer term hormonal problems were found to persist in the patients for over 30 years, although it is unclear if these effects are due to PCBs or the PCDFs which were also present in the contaminant oil.

The different PCB congeners exhibit different toxicities. Non-ortho and mono-ortho PCBs are of most concern because they exhibit dioxin-like effects with similar toxicity to PCDDs and PCDFs. They have a maximum of one chlorine atom in the ortho-position and the phenyl rings can rotate to adopt a coplanar structure (Baars et al., 2004). There are 12 of these PCBs which are referred to as 'dioxin-like'. Table 1.3 shows the Toxic Equivalency Factors (TEFs) for these PCBs. The TEF is a toxicity value assigned by the World Health Organisation (WHO) which represents the toxicity of a compound relative to 2,3,7,8-TCDD with a maximum toxicity designation of one (Safe, 1999; Van den Berg et al., 2006).

The major pathway of human exposure to PCBs is believed to be through the diet, although there is also potential for exposure via indoor air and dust (Harrad et al., 2010). Sealant materials containing PCBs have found to be a contributor to concentrations in indoor air (Balfanz et al., 1993). Since PCBs were banned in the late 1970s there has been a reduction in exposure from the diet for dioxin-like PCBs which come from sources such as incineration which has been well controlled (DEFRA, 2007). The percentage of adults estimated to exceed the TDI from the diet went from 35% in 1997 to 1.1% in 2001 (Food Standards Agency, 2003). For other non-dioxin-like PCBs, dietary exposure did not decline between 1992 and

2001, presumably due to continued emissions from their on-going use in building sealants and electrical transformers and capacitors.

Table 1.3 Dioxin like PCB Congeners and their assigned Toxic Equivalency Factors

Congener		WHO TEF[a]
77	3,3',4,4-TetraCB	0.0001
81	3,4,4',5-TetraCB	0.0003
105	2,3,3',4,4'-PentaCB	0.00003
114	2,3,4,4',5-pentaCB	0.00003
118	2,3',4,4',5-pentaCB	0.00003
123	2',3,4,4',5-pentaCB	0.00003
126	3,3',4,4',5-pentaCB	0.1
156	2,3,3',4,4',5-hexaCB	0.00003
157	2,3,3',4,4',5'-hexaCB	0.00003
167	2,3',4,4',5,5'-hexaCB	0.00003
169	3,3',4,4',5,5'-hexaCB	0.03
189	2,3,3',4,4',5,5'-heptaCB	0.00003

[a](Van den Berg et al., 2006)

PCBs have been detected in human adipose tissue in a number of studies. PCBs measured in human tissues from 11 subjects in Belgium were found to contain 29.4, 35.3, 10.6 and 11.8 ng/g wet wt in liver, muscle, kidney and brain respectively (Chu et al., 2003). PCBs 153 and 180 were found to be the major ortho-substituted PCBs accounting for 30% and 25% respectively whereas trichlorinated PCBs and lower were below the detection limit. PCBs 153 and 180 were also found to be the main congeners measured in Belgian human adipose tissue from 20 individuals (Covaci et al., 2002).

1.4.3 Fate of PCBs in the environment

Despite the restrictions on their manufacture and use, PCBs are still found widely in the environment, including in outdoor and indoor air, soil, and rivers (Jamshidi et al., 2007; Asher et al., 2007). From the available data in the early 1990s it was estimated that the bulk of the UK environmental burden of PCBs was present in soil (93.1%) followed by seawater (3.5%) and marine sediments (2.1%) (Harrad et al.,1994).

As shown in table 1.2 the lower chlorinated PCBs have higher vapour pressures than the higher chlorinated congeners. This combined with the relative abundance of the different congeners in the various commercial formulations used accounts for the predominance of PCBs such as PCB 28 and 52 in air. The higher PCB congeners also exhibit higher K_{OA} and K_{OW} values, i.e. they bind more strongly to airborne particulate matter, soil and environmental lipids and are therefore more persistent in humans and the environment. K_{OA} is dependent on temperature, and thus there is enhanced partitioning of PCBs to airborne particles and soil at colder temperatures (Harner and Bidleman, 1996). In soil, PCB persistence is positively correlated with K_{OA} and thus lower temperatures lead to longer residence times in soil. This was supported by findings in a study into the fate and persistence of PCBs in artificially contaminated soils (Ayris et al., 1999). It was found that persistence was affected by temperature, moisture content and soil organic carbon content. It was found in most instances that PCB persistence in soils was greater at lower temperatures. However it was not seen in all cases which indicates that other properties can influence persistence of PCBs. There was also observed to be greater persistence at greater soil moisture content and soil organic carbon content.

1.4.4 PCB chiral signatures

PCB enantiomers are atropisomers due to the hindered rotation about the single C-C biphenyl bond (Vetter and Schurig, 1997). Of the 209 PCB congeners, 19 exhibit chirality.

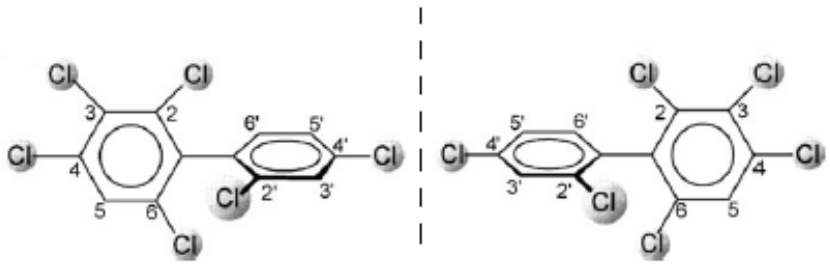


Figure 1.5: : Enantiomers of PCB 139 (Smith 2009).

The enantiomers of PCBs can be resolved chromatographically using GC/MS with a chiral stationary phase column (Robson and Harrad, 2004). Wong et al. separated successfully the enantiomers of all 19 chiral PCBs from achiral congeners using GC columns containing modified cyclodextrins (Wong et al., 2000).

The enantiomer profiles of PCBs have been used in many studies, such as those looking at air and soil (Robson et al., 2004), human tissues (Chu et al., 2003) and as a tool for determining biotransformation in biota (Warner et al., 2005; Wong et al., 2004). They have been utilised as a source apportionment tool in several environmental compartments (Lehmler et al., 2010).

Chiral signatures of PCBs have been used for source apportionment in rivers (Asher et al., 2007). It was found that the atmospheric source of PCBs to the atmosphere was likely to be local pollution rather than volatilisation from the estuary due to PCB 95 being found to be racemic in the atmosphere compared to nonracemic in water, TSM (total suspended matter), phytoplankton, and sediments. Chiral signatures were also used to investigate the

biotransformation of PCBs in Arctic biota (Warner et al., 2005). It was found that there was stereospecific biotransformation in some species including some seabirds and ringed seals.

PCBs chiral signatures have also been used to provide insights into their behaviour in soil, plants and air. Along with other POPs, PCBs in grass are believed widely to originate primarily from the atmosphere, with inputs from soil considered negligible (McLachlan, 1996). However, it has been suggested recently that soil contamination may exert a greater influence on the concentrations of PCBs in grass than hitherto realised. While chiral signatures in soils and grass were similarly non-racemic, those found in "bulk" air sampled at ca 1.5 m above the surface were racemic (Harrad et al, 2006). The authors hypothesised this implied that levels in grass arose predominantly due to foliar uptake of PCBs volatilised from soil. They suggested that at background soil concentrations, the impact of such volatilisation on concentrations in air is significant only at the soil:air interface, thereby providing a source of the non-racemic signature in grass, while explaining why chiral signatures in "bulk" air sampled at 1.5 m height are racemic (Harrad et al., 2006). Furthermore, it has been found that PCBs 95 and 149 in indoor and outdoor air have similar racemic chiral signatures, suggesting that ventilation of indoor air contributes more to levels in outdoor air than volatilisation from soil (Jamshidi et al, 2007). This is because chiral signatures of PCBs 95 and 149 in top soil are non-racemic. Moreover, such non-racemic signatures in soil suggests that degradation occurs in soil at concentrations much lower than previously thought (Robson et al., 2004). The idea that volatilisation from soil may be a major route for POPs entering grass is thus an area that needs further investigation.

Another potential reason for non-racemic signatures of PCBs in plants could be biotransformation within the plant itself. A recent study by Zhai et al., suggests that PCB 95 is

enantioselectively biotransformed in whole Poplar plants (Zhai et al., 2011). There was found to be a deviation from racemic of PCB 95 in the middle and bottom xylem of the poplar plant over a 20 day exposure. This study suggests that PCB 95 was enantioselectively metabolised within the plant. While of substantial interest, it must be stressed that these findings are related only to poplar plants exposed hydroponically to PCB 95, that there is substantial inter-species variation in the ability of plants to translocate PCBs from root to foliage, and furthermore that translocation of PCB 95 in poplars is minimal compared to that observed for less chlorinated congeners like PCB 3 (Zhai et al, 2011).

1.5 Hexabromocyclododecane (HBCD)

1.5.1 Introduction

HBCD is a brominated flame retardant being found in increasing levels in the environment, owing to its use as a flame retardant in thermal building insulation, upholstery textiles and electronics (Covaci et al., 2006). HBCD has been produced since the 1960s and is currently the most widely used additive brominated flame retardant (Marvin et al., 2011). The current known production of HBCD is approximately 28,000 tonnes with China producing 9,000-10,000 tonnes and Europe and US producing 13,426 tonnes in 2009 (UNEP, 2011). There is limited information on HBCD production for other countries. The total amount of HBCD used worldwide in 2001 was 16,700 tonnes with the majority (9,500 tonnes) sold in the EU (BSEF). The volume of HBCD used in the EU in 2006 was approximately 11,580 tonnes, with 6,000 tonnes coming from imports (UNEP 2011). HBCD is under consideration for inclusion as a POP according to the Stockholm convention on POPs. In May 2009, it was included in ECHA's (European Chemical Agency) recommendation list of priority substances

to be authorised under REACH and the US Environmental Protection Agency (EPA) has begun a review on HBCD to be completed in 2012 (BSEF).

Table 1.4 shows the physicochemical properties of the HBCD commercial product (commonly expressed as ΣHBCDs - a mixture of different diastereoisomers, principally α, β, and γ-) and of the three predominant diastereoisomers individually. HBCD is lipophilic with a log K_{ow} of 5.6 (Birnbaum and Staskal, 2004). It is an additive flame retardant, being mixed in to rather than bound chemically to the material within which it is incorporated, making it likely to leach into the environment during use and disposal (Law et al., 2005). HBCD melts over the temperature range 185-195^{0}C, decomposes at temperatures exceeding 240^{0}C and the isomers are liable to rearrangement above 160^{0}C (D'Silva et al., 2004; Birnbaum and Staskal 2004).

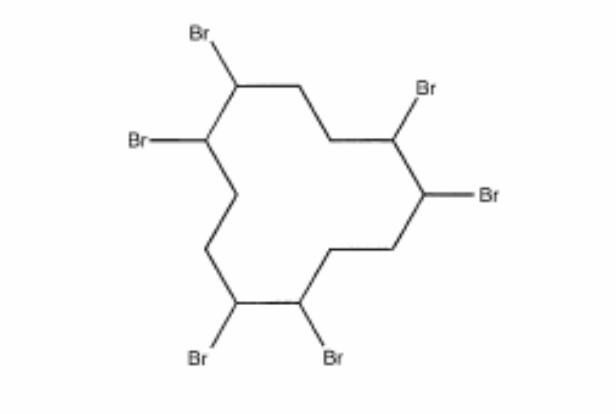


Figure 1.6: Structure of HBCD (Cariou et al., 2005)

HBCD is an aliphatic brominated, cyclic compound, which has 16 potential stereoisomers. These are 6 diasteromeric pairs of enantiomers and 4 *meso* forms (optically inactive isomers that have an internal plane of symmetry) (Law et al., 2005). Currently 8 stereoisomers have

been identified in the commercial formula which are the three pairs of enantiomers assigned α, β, and γ, and low levels of *meso* forms δ and ε (Morris et al., 2004). A low melting point technical product was found to consist predominantly of γ-HBCD (81.6%) followed by α-HBCD (11.8%), β-HBCD (5.8%), δ-HBCD (0.5%) and ε-HBCD (0.3%) (Law et al., 2005). Another study has reported on a technical mixture consisting of predominantly γ-HBCD at levels exceeding 98% (Ryan et al., 2006).

Table 1.4 Physicochemical properties of HBCD technical mixture (KEMI (National chemicals inspectorate), 2007)

Property	Value
Chemical formula	$C_{12}H_{18}Br_6$
Molecular weight	641.7 g mol^{-1}
Boiling point	Decomposes at >190 C
Melting point	179-181 ºC α-HBCD 170-172 ºC β-HBCD 207-209 ºC γ-HBCD
Density	2.24 g cm^{-3}
Vapour pressure	6.27 x 10^{-5} Pa at 21 ºC
Water solubility	7.60 x 10^{-8} mol L^{-1} α-HBCD 2.29 x 10^{-8} mol L^{-1} β-HBCD 0.33 x 10^{-8} mol L^{-1} γ-HBCD 1.03 x 10^{-7} mol L^{-1} ∑HBCD
n-octanol/water partition coefficient	Log K_{ow} = 5.62 (ΣHBCD) 5.07 ± 0.09 α-HBCD 5.12 ± 0.09 β-HBCD 5.47 ± 0.10 γ-HBCD

GC/MS has been used in the past to determine total HBCD concentrations, although this is not a feasible method for quantifying individual diastereoisomers and enantiomers as HBCDs

are liable to rearrangement at high temperatures. As a consequence, LC/MS is an ideal and commonly used analytical technique particularly for measuring the diastereoisomers and enantiomers.

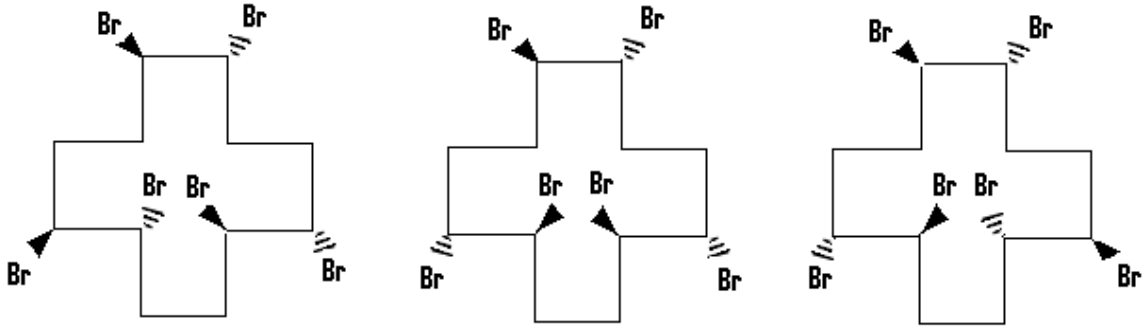


Figure 1.7: Left to right, α, β, and γ-HBCD stereoisomers

The diastereoisomers elute in the order α, β, γ on a C18 column and α, γ, β on a C30 column. Dodder et al. found that analysis of HBCD enantiomers was affected significantly by matrix effects (Dodder et al., 2006). These problems can be minimised by using isotopically-labelled HBCD isomers as internal standards (Tomy et al., 2005).

1.5.2 Health effects and human exposure to HBCDs

There are limited data on the toxicological effects of HBCDs. Although their acute and chronic toxicity appears low, many studies have only been conducted using the commercial mixture thereby failing to account for exposure to matrices displaying very different diastereoisomer pattern, e.g. via dust (Law et al., 2005). Although the health effects of HBCD are not yet fully understood there have been studies which have indicated that HBCD may cause detrimental effects to health. For example, there is potential for endocrine disruption in animals and humans (Yamada-Okabe et al., 2005). Moreover, studies on the neurotoxicological effects have found that HBCD has an effect on the uptake of the

neurotransmitters dopamine, glutamate and γ-amino-*n*-butyric acid in rats (Mariussen and Fonnum, 2003), and HBCD may also cause cancer via a non-mutagenic mechanism (Helleday et al., 1999; Ronisz et al., 2004).

There is little information on the toxicological effects of the individual HBCD diastereoisomers and enantiomers. Zhang et al., found that the HBCD stereoisomers exhibit different toxicity to Hep G2 cells with cytotoxicity of γ-HBCD ≥ β-HBCD > α-HBCD and the (+)-enantiomers exhibited greater toxicity than the (-)-enantiomers (Zhang et al., 2008). This suggests that the toxicity of HBCDs should be evaluated as individual enantiomers not as total HBCDs.

Non-occupational human exposure to HBCDs occurs via ingestion of food and indoor dust, as well as via inhalation of indoor air (Abdallah et al., 2008c). Table 1.5 shows exposure estimates for adults and toddlers. The potential significance of exposure via dust ingestion was illustrated by a study that examined concentrations of HBCDs in serum, diet and indoor dust collected from 16 participants. The study found that exposure via dust correlated with serum levels but that dietary exposure did not (Roosens et al., 2009). The exposure of children and toddlers to HBCDs from dust is higher due to the greater time they spend on the floor and the hand to mouth action common to babies and toddlers. Exposure of U.K. children to HBCD from classroom dust was found to be greater than that of U.K. adults via office dust (Harrad et al., 2010). A study of exposure due to ingestion of house, office and car dust based on 21 UK adults, revealed an average exposure of 48 ng day^{-1} ∑HBCD with the diastereoisomers accounting for 35%, 11% and 54% for α-, β- and γ-HBCD respectively (Abdallah and Harrad, 2009). This study found that house dust is the major contributor to personal exposure via dust ingestion due to the time spent at home; although on average

exposure from car dust was found to make a higher contribution to ∑HBCD than office dust due to significantly higher concentrations in car dust.

Table 1.5 Estimates of average human exposure to HBCD (ng/day)

	Adult				Child (6-24 months)			
	α-HBCD	β-HBCD	γ-HBCD	∑HBCD	α-HBCD	β-HBCD	γ-HBCD	∑HBCD
Air[a]	1.2	0.6	3.2	5	0.2	0.1	0.6	1.0
Dust[ab]	46.6	15.3	69.6	131.5	144.7	47.2	212.0	403.8
Diet[c]	203	105	112	413	120	57	67	240

[a] data from Abdallah et al., 2008c
[b] Average dust intake scenario = 20 mg day^{-1} for adults and 50 mg day^{-1} for toddlers
[c] Food Standards Agency, 2006

In terms of dietary exposure fish is thought to be a major source particularly in countries such as Sweden which has a high fish consumption (Covaci, 2006). HBCD dietary intake was estimated in Sweden via the market basket method in 2005, finding that the major dietary intake was via consumption of fish (65%), followed by dairy products (24%) and meat (10%) (Törnkvist et al., 2011). The major intake of HBCDs in a study of foods from the UK was found to be milk, fresh fruits and fish (Food Standards Agency, 2006). A study of dietary intake of HBCD in Belgium found meat to be the major source for this country (Goscinny et al., 2011).

1.5.3 HBCD diastereoisomers

HBCD has a complex stereochemistry. HBCD stereoisomer profiles found in the environment frequently deviate from those found in the commercial mixture in which the predominant isomer is γ-HBCD. This is particularly noticeable in biota but has also been seen in other environmental samples, including air, dust and soil (Janák et al., 2005; Hoh and Hites, 2005; Abdallah et al., 2008a; Yu et al., 2008a). Diastereoisomer-specific data for HBCDs reveals

profiles in sediments similar to those present in the commercial product with gamma the major isomer (Marvin et al., 2006; Harrad et al., 2009b). Yu et al found that the ratios of the three diastereoisomers in three soil samples showed different patterns with two samples showing similarities with the commercial product and one showing a dominance of the alpha isomer (Yu et al., 2008a).

Possible explanations for these deviations could be

1. Thermal isomerisation which could occur during the processing of HBCDs
2. Faster degradation of γ-HBCD and β-HBCD compared to α-HBCD.
3. Bioisomerisation from γ-HBCD and β-HBCD to α-HBCD
4. Differences in the solubility of the diastereoisomers

Thermal isomerisation can occur during the incorporation of HBCD into materials as they are liable to arrangement at temperatures exceeding 160^{o} C (Covaci et al., 2006). It was observed in flame retardant treated textiles that there was a higher proportion of α-HBCD compared to the commercial mixture, which suggests that there could be isomerisation during the heating process used to combine the flame retardant to the material (Kajiwara et al., 2009). This could also account for the difference in diastereoisomer profile in dust compared to the commercial mixture. A study looking at the mechanism of γ- to α-HBCD isomerisation concluded that α-HBCD is more thermodynamically stable and that it can be formed from γ-HBCD at temperatures exceeding 100^{0} C (Heeb et al 2008). There is a complex interconversion of the diastereoisomers at elevated temperatures (Köppen 2008). It was found that β-HBCD and γ-HBCD degraded more rapidly than α-HBCD by an estimated factor of 1.6 and 1.8, respectively (Gerecke et al., 2006).

There is evidence of photolytically induced change from γ-HBCD to α-HBCD in dust samples exposed to light over a one week period (Harrad et al., 2009a). This study also exposed to light, standard solutions of individual HBCD diastereoisomers and found that each diastereoisomer isomerised to produce the other two but most strongly favoured a shift from γ-HBCD to the α-HBCD isomer. There was also observed to be a net loss of ΣHBCDs as a result of degradation.

Biota samples usually contain α-HBCD at the highest levels (Morris et al., 2004; Janák et al., 2005; Tomy, 2004). This could be due to the differences in hydrophobicity of the diastereoisomers or the preferential metabolism of one diastereoisomer over another. Abdallah and Harrad found human milk samples to also contain predominantly α-HBCD (Abdallah and Harrad, 2011). Another study found human milk to contain predominantly γ-HBCD (54-100%) (Eljarrat et al., 2009). Roosens et al., found serum samples to contain only α-HBCD with similar results also seen in serum samples by Weiss et al. with α-HBCD accounting for 97–99% of ∑HBCDs in blood from 53 participants (Roosens et al., 2009 ; Weiss et al., 2006). It has been suggested that the predominance of α-HBCD in human samples could be due to the ingestion of dust which has been observed to contain higher levels of α-HBCD than is found in the commercial mixture (Abdallah et al., 2008a). It could also be due to *in vivo* diastereoisomer specific metabolism. Preferential metabolism has been reported of γ-HBCD and β-HBCD by cytochrome P450, which could explain the high levels of α-HBCD seen in biota samples and human milk samples (Zegers et al., 2005). However Esslinger et al., found the relative degradation rates by phase I metabolism of the diastereoisomers would not favour an enrichment of α-HBCD in biota (Esslinger et al., 2011). They were also able to determine distinct patterns of monohydroxylated derivatives for both

α- and γ-HBCD as a result of phase I metabolism. It was observed for fish samples that there were more α-HBCD derivatives compared to γ-HBCD, which is unexpected if γ-HBCD was being preferentially metabolised. These results suggest it may be a complex combination of processes.

The solubility of the individual isomers can also be a factor particularly in aquatic environments. The α-HBCD isomer has been found to be more water soluble than β- and γ-HBCD. Hunziker et al., determined the water solubility of the three diastereoisomers to be 48.8, 14.7 and 2.08 μg/L for α-, β- and γ-HBCD respectively (Hunziker et al., 2004). This may have an implication for levels in biota as α-HBCD will have increased bioavailability. It was found in an *in vitro* study of the bioavailability of HBCDs in the human gastrointestinal tract (GIT) that γ-HBCD was less bioavailable than α- and β-HBCD, likely due to γ-HBCD's lower water solubilty (Abdallah et al., 2009). Despite the deviation from the diastereoisomer profile observed in dust in the human GIT samples it was still predominantly γ-HBCD which suggests the predominance of α-HBCD found in biota may be due to *in vivo* biochemical processes.

There has also been observed to be differences in the vapour pressures of the diastereoisomers with γ-HBCD being found to be an order of magnitude lower than that of the other two diastereoisomers with values of 1.05×10^{-8}, 5.82×10^{-9} and 8.39×10^{-11} Pa at 25 ^{o}C reported for α-, β- and γ-HBCD respectively (Kuramochi and Sakai, 2010). This could affect the way the individual diastereoisomers partition between the solid and gaseous phase.

1.5.4 Fate of HBCDs in the environment

HBCD has been found in most environmental media and is a ubiquitous contaminant being found in many different matrices including soils, sewage sludge, air, river sediments, fish, house dust, human milk and remote areas due to long range transport (Covaci et al., 2006, Law et al., 2005). They are highly bioaccumulative (Wu et al., 2011). HBCDs are still manufactured and in current use and have been found at increasing levels in some areas of the environment. HBCD has become more of a focus particularly in the EU as restrictions have been placed on other flame retardants such as PBDEs (Law et al., 2008). For example a rapid increase in concentration has been observed in sediment core samples from Switzerland taken from the mid 1980s until 2001 (Kohler et al., 2008). In a study of ringed seals in East Greenland there was found to be a significant annual increase in HBCD levels of 6.1% in samples from 1986 to 2008 (Vorkamp et al., 2011). A study of the blubber of 85 porpoises from the UK between 1994 and 2003 found there to be a sharp increase in HBCD from 2001 (Law et al., 2006). However, it has been found more recently that the levels in cetaceans from the UK have been declining (Law et al., 2008). This may be in response to restrictions on HBCD production and use.

The low volatility and low water solubility of HBCDs, means that they tend to be sorbed onto solid matter in the environment. Studies that have measured the distribution of HBCD between the gas and particle phase in outdoor air have found them to be predominantly in the particulate phase (Hoh and Hites, 2005; Yu et al., 2008b). Conversely HBCD measured in indoor air has been found predominantly (~65%) in the gas phase (Harrad and Abdallah, 2007). This is likely because of the higher temperatures indoors. Yu et al., measured HBCD in outdoor air from four urban sites and found that 69.1-93.3% of HBCD was sorbed onto particulate matter (Yu et al., 2008b). This study also found there to be a difference in the

relative abundances of the three diastereoisomers between the gas and particle phase with α- and γ-HBCD being found at higher percentage in the particle phase compared to the gas phase and β-HBCD being found predominantly in the gas phase.

1.5.4.1 HBCDs in air and soils

HBCD has been detected in outdoor air from the USA between 0.16 and 11 pg ΣHBCDs/m^3 (Hoh and Hites, 2005), from Sweden in both rural and urban locations at concentrations between 2 and 610 pg ΣHBCDs/m^3 (Remberger et al., 2004), and in two studies in China where concentrations of ΣHBCDs were found to fall between 0.69 and 3.09 pg/m^3 (Yu et al., 2008a, Yu et al., 2008b). A study on HBCD in air from Birmingham, UK found outdoor air to contain 37±2 pg/m^3 ∑HBCD compared to much higher concentrations in indoor air with average ∑HBCD found to be 250pg/m^3 in homes, 180 pg/m^3 in offices and 900 pg/m^3 in public microenvironments (PME) (Abdallah et al., 2008c).

There are limited data available on the levels of HBCDs in soils. This is an important medium as soils provide a potential route of HBCD into the terrestrial food chain. Due to the hydrophobic properties of HBCDs, they can bind strongly to soil and sediments. High levels of ∑HBCDs were found close to an extruded polystyrene (XPS) producing plant in Sweden with levels ranging from 140-1300 ng/g dry weight (Remberger et al., 2004). Soils collected near to HBCD processing plants in Belgium and Germany were also found to have high concentrations of ∑HBCD ranging from 111-23200 ng/g dry weight (Petersen et al., 2004). One study in China found soil contained 1.7-5.6 ng ΣHBCDs/g dry weight (Yu et al., 2008a). However, a second Chinese study found lower levels in soil ranging from 1.2-1.8 pg ∑HBCDs/g (Meng et al., 2011).

Table 1.6 HBCD levels in sediments, soils and air

	Sampling site		∑HBCD	Alpha	Beta	Gamma	Reference
Sediment	Detroit river	ng/g dry wt.	<0.075-3.7	<0.025-1.9	<0.025-0.28	<0.025-2.3	Marvin et al. 2006
	England	ng/g dry wt.	<2.4-1680				Morris et al., 2004
	Scheldt basin, Belgium	ng/g dry wt.	<0.2-950				
	UK Lakes	ng/g dry weight	(0.88-4.8)	(0.11-0.62)	(0.064-0.50)	(0.66-3.8)	Harrad et al., 2009b
Soil	Sweden	ng/g dry weight	567 (140-1300)				Remberger et al., 2004
	Belgium/ Germany	ng/g dry weight	4292 (111-23200)	618	321	3353	Petersen et al., 2004
	Chongming island , China	ng/g dry weight	0.023 ±0.019	0.0055 ±0.0047	0.0012 ±0.0013	0.017 ±0.014	Meng et al., 2011
	Guangzhou, China, Urban	ng/g dry weight	1.7-5.6				Yu et al., 2008a
Air	Guangzhou, China, Urban	pg/m^3	1.2-1.8				Yu et al., 2008a
	Guangzhou, China, Urban	pg/m^3	0.69-3.09				Yu et al., 2008b
	Indoor air, Homes, UK	pg/m^3	250 (67-1300)	59 (14-430)	22 (5-54)	170 (39-710)	Abdallah et al., 2008c
	Offices, UK	pg/m^3	180 (70-460)	43 (18-87)	24 (14-34)		
	Public microenvironments, UK	pg/m^3	900 (820-960)	250 (180-400)	28 (19-46)	550 (360-690)	
	Outdoor air, UK	pg/m^3	37 (34-40)	3.0 (2.3-3.7)	1.1 (0.9-1.2)	33 (31-35)	

1.5.5 HBCD chiral signatures

The use of chiral signatures to help elucidate aspects of the environmental fate and behaviour of HBCD has attracted attention recently. The three HBCD diastereoisomers α, β, and γ exist as enantiomer pairs.

HBCD enantiomers have been determined using LC/MS/MS with a chiral column (Law et al., 2005). Heeb et al. were able to separate 8 out of the potential 16 HBCD stereoisomers using LC/MS/MS (Heeb et al., 2005). The chromatographic separation of the enantiomers on a chiral column can be seen in figure 4 and shows that the first eluted HBCD enantiomers of each distereomers are (-) α, (-) β and (+) γ (Janák et al., 2005).

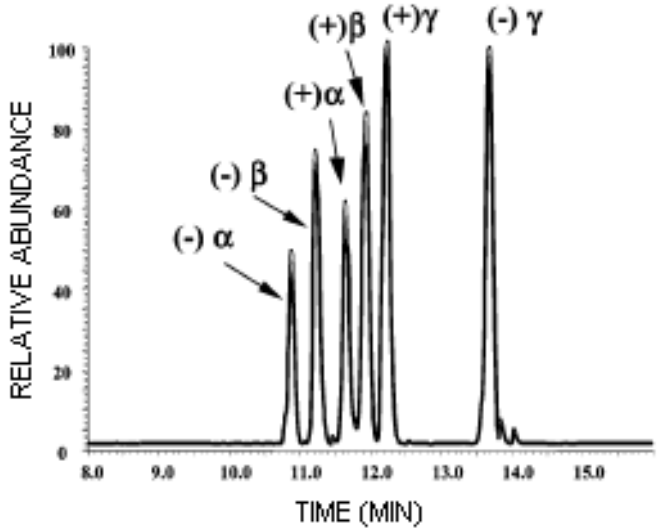


Figure 1.8: Chromatogram showing the elution order of the enantiomers of α-, β-, and γ-HBCDs (Janák et al., 2005)

A study into the anaerobic degradation of HBCD found there to be no evidence that degradation of HBCD was an enantioselective process (Gerecke et al., 2006). Harrad et al.,

found no significant change in the enantiomer fractions of dust exposed to light despite seeing a shift in the diastereoisomer profile and degradative loss (Harrad et al., 2009a).

Janák et al. determined enantiomer fractions of α, β, and γ-HBCD in fish samples, where it was found there was enrichment of the (+) α-HBCD in liver samples of two fish species (Janák et al., 2005). A recent study has also found that there is significant enrichment of (-)-α-HBCD in samples of UK human milk with an average EF of 0.29 (Abdallah and Harrad, 2011). Similar results were also reported for Spanish human milk (Eljarrat et al., 2009). Moreover, in serum samples from Belgium there was found to be substantial enrichment of (–)-α-HBCD with an EF of 0.28 ± 0.02 suggesting *in vivo* metabolism (Roosens et al., 2009).

Table 1.7 shows the half lives of HBCD isomers after incubation with rat liver microsomes (Esslinger et al., 2011). It shows there to be significant difference in half life between the different stereoisomers and also difference between the enantiomers for α-HBCD and γ-HBCD, where (-)-α-HBCD and (+)—γ-HBCD both show significantly longer half lives compared to their corresponding enantiomer (Esslinger et al., 2011). This indicates that metabolism could play a role in the deviation from racemic of the enantiomers observed in biota samples.

Table 1.7 Half lives ($T_{1/2}$) of HBCD isomers after incubation with rat liver microsomes (Esslinger et al., 2011)

HBCD isomer	$T_{1/2}$ (mins)
(-)-α	24.4
(+)-α	14.1
(-)-β	6.32
(+)-β	6.35
(-)-γ	32.3
(+)-γ	11.6

Guerra et al. found an enrichment of (+)-α-HBCD and (+)-γ-HBCD in sediment samples (Guerra et al., 2008). There was found to be an enrichment of (-)-α-HBCD in air from a city in south China suggesting there could be soil-air exchange of HBCDs (Yu et al., 2008b). This is an important finding as it may indicate that chiral signatures of HBCDs could be used to determine the relative contribution of volatilisation from soil to atmospheric concentrations as has been done previously for PCBs. There are gaps in knowledge as to how HBCD transfers throughout the environment and in the context of this project, enantiomer signatures could potentially be used to determine the pathways via which HBCDs could enter plants.

1.5.6 Degradation products of HBCD

The degradation products of HBCD have recently attracted interest although there is currently limited information on these compounds and mechanisms by which they are formed. Two degradation products of interest are PBCDs (pentabromocyclodododecenes) and TBCDs (tetrabromocyclododecadienes) which have been identified in dust samples, human milk, and sediments (Abdallah et al., 2008b, Abdallah and Harrad 2011, Harrad et al., 2009b). PBCDs have also been observed in chicken eggs and fish using gas chromatography (Hiebl and Vetter, 2007). These degradation products could be forming in the environment through bio-, photolytic, and thermal degradation or may be due to thermal degradation of HBCD during its production and incorporation into materials.

Esslinger et al. were able to identify hydroxylated metabolites of HBCD as result of phase I metabolism (Esslinger et al., 2011). No debrominated metabolites of TBCD or PBCD were detected. Brandsma identified four different groups of hydroxylated HBCD metabolites in Wistar rats exposed to HBCD (Brandsma et al., 2009).

The mechanism by which HBCD degrades is not fully understood. A study into biodegradation of HBCD in sediment and sewage sludge suggested that HBCD degrades via the sequential loss of Br_2 to give tetrabromocyclododecene (TBCDe), dibromocyclododecadiene, and cyclododecatriene (Davis et al., 2006). Abdallah et al., found that in the case of dust the loss of HBr is the major mechanism for the degradation of HBCDs (Abdallah et al., 2008b). This study found there to be four chromatographic peaks attributable to PBCDs, as shown in figure 1.9 and two peaks assigned as TBCDs. It was found in surficial sediments from English lakes that elimination of HBr was also the suggested mechanism via which the HBCDs were degraded, as seen for dust (Harrad et al., 2009b). In this study all sediment samples were found to contain TBCDs and four samples contained PBCDs with the number of peaks corresponding to those found in dust samples.

A recent study found that exposing indoor dust samples to light over the period of a week resulted in a slow degradative loss of HBCDs to PBCDs (Harrad et al., 2008). This process was seen in the absence of light but was more evident in its presence showing that photolytic degradation of HBCD occurs. It was also seen in a study into the transformation of HBCD that biotransformation processes accelerated the loss of HBCDs, although degradation products were not detected in this study (Davis et al., 2005).

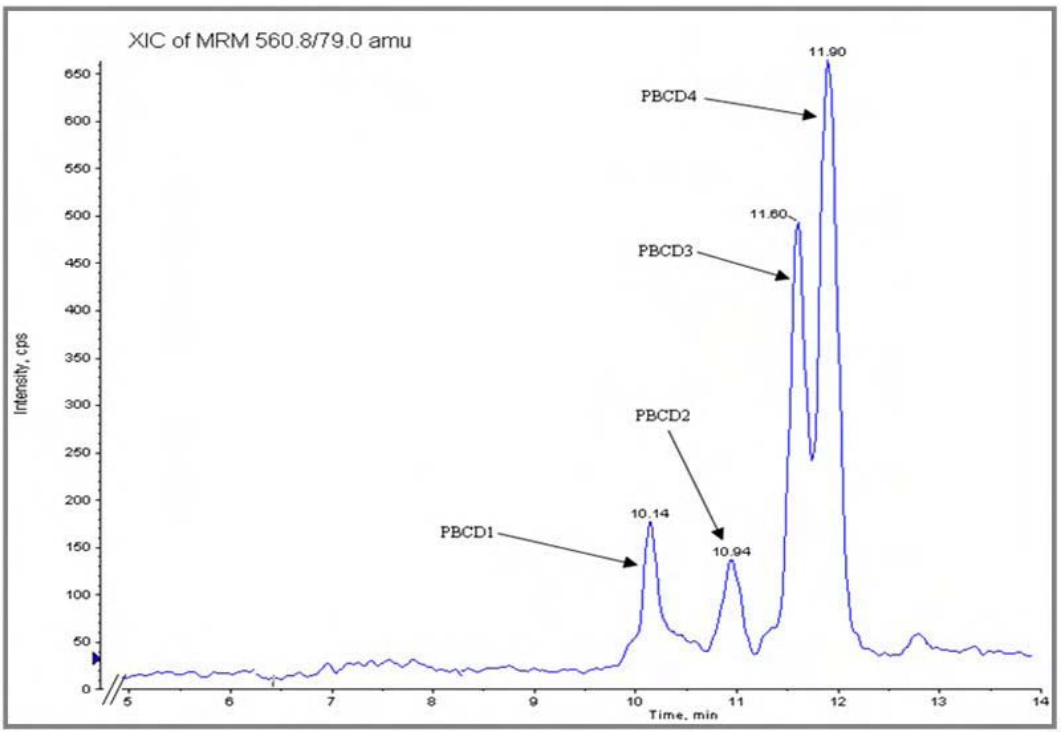


Figure 1.9: Chromatogram of PBCDs in dust (Harrad et al., 2009a)

The PBCD and TBCD degradation products have also been semi-quantitatively determined in human milk samples (Abdallah and Harrad, 2011). The isomer profiles were found to differ from the dust with only three chromatographic peaks assigned as PBCDs observed, suggesting that *in vivo* metabolism of the compounds could be occurring.

There is limited knowledge on these degradation products, the toxicological effects and the mechanisms via which they are formed. As these degradation products have only recently been identified the toxicological effects are not yet fully understood. However, a recent study has suggested that PBCDs have significantly stronger binding affinity than HBCDs for the endocrine human transthyretin receptor (hTTR) (Weber et al., 2009).

The fact that PBCDs and TBCDs have the potential to cause detrimental effects to health suggests that human exposure should be determined for these compounds. As with other details on these compounds the information available is limited. Abdallah and Harrad (2009) determined typical human exposure to ∑TBCDs and ∑PBCDs via the ingestion of dust to be 0.2 ng/day and 1.4 ng/day respectively. This was significantly lower than typical exposure to HBCD of 48 ng/day. However, extensive variation between the exposures of individuals, meant that exposure of one participant to ∑PBCDs exceeded the exposure to ∑HBCDs for 85% of the other participants.

1.6 Conclusions

There are a number of mechanisms by which POPs enter plants from air and soil which is dependent on a number of factors including the physicochemical properties of the POP, the species of the plant and the climate conditions. Air to plant transfer is considered the major route via which POPs are transferred into plants.

Recently chiral signatures have been utilised as a means of determining these pathways as enantiomers can react differently with biological systems. Commercial mixtures contain an equal ratio of each enantiomer and therefore when deviations from this are found in environmental samples due to enantioselective degradation it can be used as a means of tracing the origins of these compounds. Although it has been the consensus that the main pathway of POPs into plants was from air, recent preliminary work using chiral signatures has suggested that this may not the case. This is an area therefore that requires further investigation.

PCBs and HBCDs are POPs of interest due to their persistence in the environment and adverse effects to human health. Both PCBs and HBCDs exhibit chirality and are ubiquitous in the environment, which makes them ideal candidates for the use of chiral signatures as a means of determining their pathways into plants. Chiral signatures of PCBs and related organochlorine contaminants have been used in many previous studies to determine pathways and HBCD chiral signatures have the potential to be used in the same way. Degradation products of HBCD have also gathered recent interest. Although they are currently found at much lower levels than their parent compound they also have the potential to cause adverse effects to human health. There are currently limited data on these compounds and the mechanisms via which they are formed.

1.7 Aims and objectives of the project

The main aim of the project is to determine the uptake pathways of HBCDs and PCBs into plants using chiral signatures to establish whether they enter grass primarily from the atmosphere or from soil. We hypothesise from previous work using chiral signatures that volatilisation from soil is an important source of POPs in grass.

The main objectives are:

1. To determine concentrations of PCBs found in soil, grass and air at graduating heights from the soil surface.
2. To determine the chiral signatures of PCBs 95 and 136 in these samples to test the hypothesis that volatilisation of soil is an important source of these contaminants in grass.
3. To determine whether HBCDs in soils show enantioselective degradation, and if so to see how chiral signatures in grass are comparable to those in air close to the soil surface and soil.

4. To determine HBCD concentrations from soils from different sites in the UK.
5. To examine diastereoisomer profiles from these soils as well as in air and grass samples and compare them to those seen in the commercial mixture. This will test the hypothesis that the patterns in air, soil and grass will shift further towards α-HBCD than in indoor air and dust because of the greater potential for photolysis in the outdoor environment.
6. To determine if the degradation products PBCDs and TBCDs are present in soil samples from the UK, thereby evaluating whether the main degradation pathway of HBCD is similar to that seen in sediment and dust samples.
7. to determine the degradation products in textile samples treated with HBCD which have been stored in the presence and absence of light to examine the role of photolysis in the degradation of HBCD in such applications.
8. To determine the diasteroisomer profiles of HBCDs in a number of Australian soils to test the hypothesis that – compared to UK soils - there will be an enhanced shift to α-HBCD due to the greater potential in Australia for photolysis and thermal degradation.
9. To test the hypothesis that the higher solar irradiance and temperatures (with associated microbial activity) experienced by Australian soils, will lead to chiral signatures and patterns of HBCD degradation products that are distinct from those found in UK soils.
10. To construct a preliminary environmental budget for HBCDs in the UK environment.

CHAPTER 2: SAMPLING AND ANALYTICAL METHODOLOGY

2.1 Synopsis

This chapter describes the sampling methods and the analytical techniques used for this project. The analytical method for measuring HBCD in air samples was from an existing method developed by Abdallah et al., (Abdallah et al., 2008a). The method for measuring HBCD in soil was developed from this method over the course of the project. The analytical method for measuring PCBs in air, soil and grass samples was adapted from existing methods (Ayris et al., 1997).

2.2 Sampling strategy

2.2.1 Location

Sampling in Birmingham took place at the Elms Road Observatory Site (EROS) location on the University of Birmingham campus. This site is approximately 3 km south west from Birmingham city centre. Birmingham is the second largest city by population in the UK. A map of the University in relation to Birmingham is shown in figure 2.1 and the location of EROS on the university campus is shown in figure 2.2.

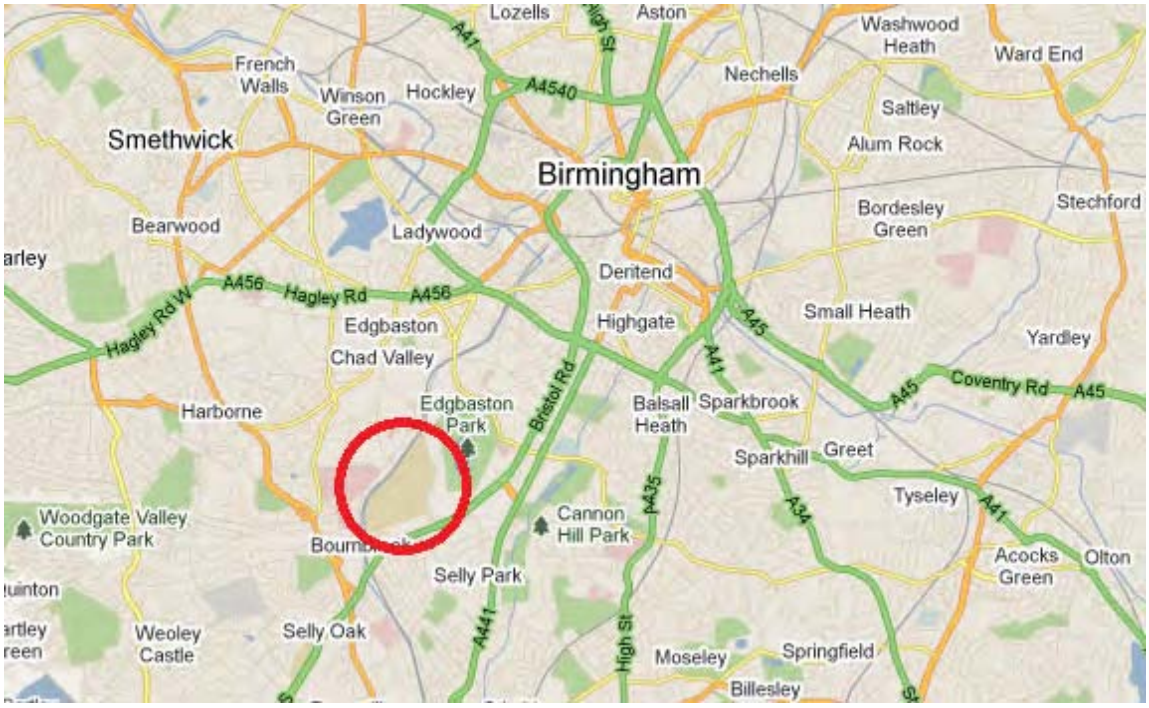


Figure 2. 1: Birmingham University Location

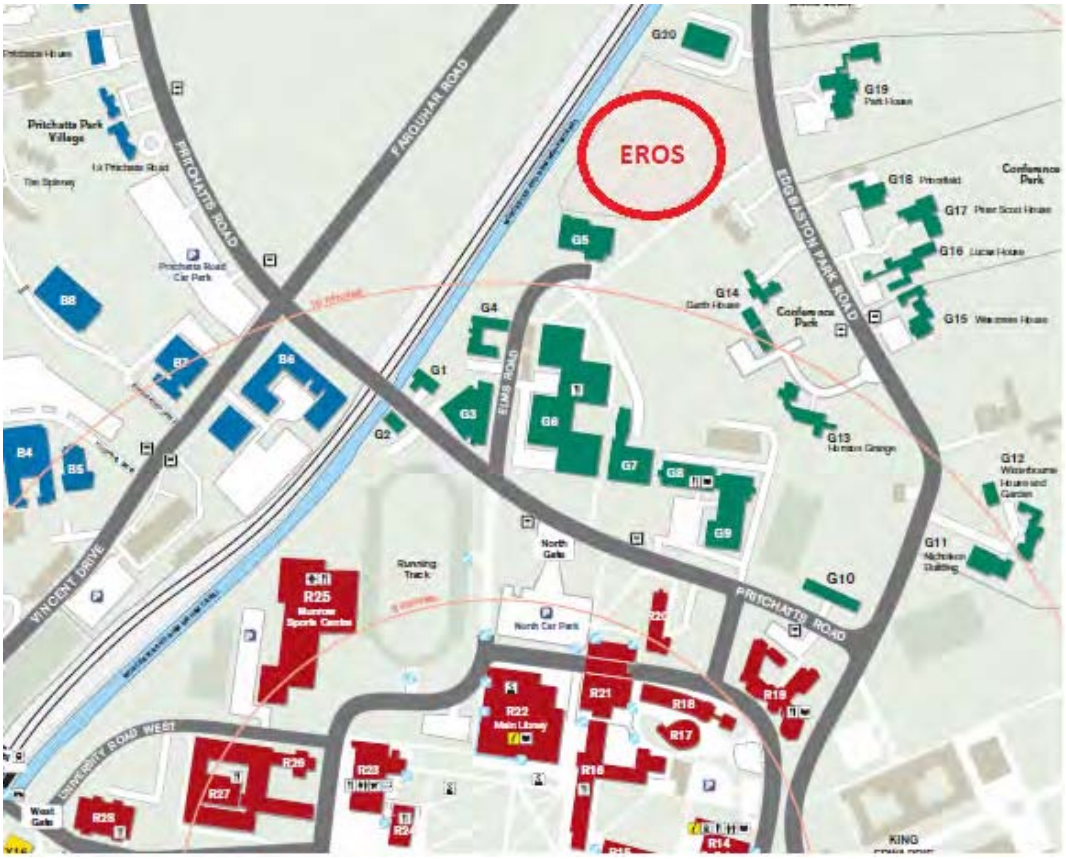


Figure 2. 2: EROS location on the University of Birmingham campus

2.2.2 Air sampling

Air was sampled using passive air samplers at the EROS location on the University of Birmingham campus.

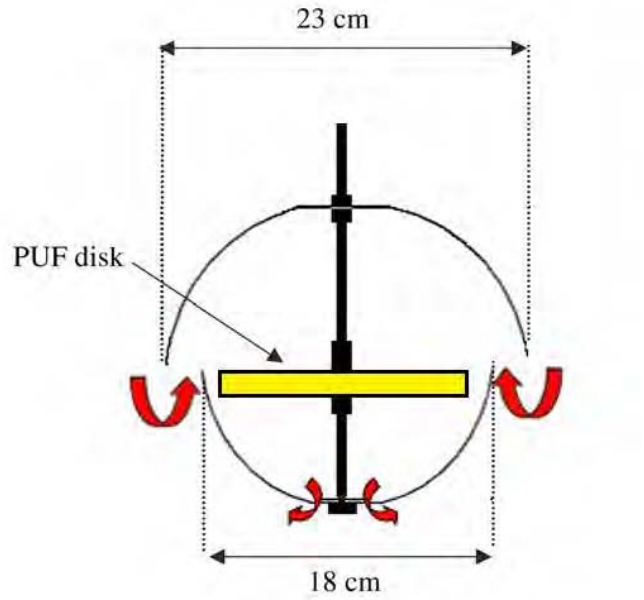


Figure 2. 3: Passive air sampler configuration used to monitor HBCDs

The sampler comprised of two different sized stainless steel shelters which housed one or two polyurethane foam (PUF) discs. For monitoring atmospheric concentrations of HBCDs, one PUF was placed in the shelter as shown in figure 2.3. For monitoring PCBs, two PUF discs were used in each sampler, in line with the method used in a previous study by Evans (Evans, 2007). Following sampling, the two discs were combined for analysis as a single air sample.

Figure 2. 4: PAS at EROS

The PUF discs were washed with distilled water to remove any adhering particles, dried and then precleaned in a soxhlet apparatus with DCM for 8 hours. They were then stored wrapped in foil inside resealable plastic bags until ready to be transferred to the air samplers. Once in the sampler they were spiked with a sampling evaluation standard (SES) and kept in re-sealable airtight plastic bags while being transported to the sampling site. After the sampling period the samplers were taken down, resealed in the airtight plastic bags and stored in a cold room (4 ˚C) where they remained until they were ready for extraction.

2.2.2.1 HBCD air sampling

Sampling of air at graduating heights was done at five heights above the soil surface. In 2008 HBCDs were determined in air samples collected at heights of 10 cm, 54 cm, 100 cm, 122 cm, and 142 cm for 14 and 28 days. HBCD was also measured in outdoor air at every 14 days for 4 months in 2010 at 1.5 m height from the ground beginning on 26th March, with samples

taken at t = 14, 28, 42, 56, 70, 84, 98 and 112 days. Two samplers were used and the PUFs combined to ensure detectable amounts of HBCDs.

2.2.2.1 PCB air sampling

For PCBs, two campaigns were conducted: one in 2009, the other in 2010. In 2009, sampling began on 3rd June, with samples of soil, grass and air taken at t = 15, 29, 44, 58, 72, 85, 100, and 114 days. Sampling in 2010 began on 26th March, with samples taken at t = 14, 28, 42, 56, 70, and 84 days. The heights used in both campaigns were 3 cm, 10 cm, 40 cm, 90 cm and 130 cm. Sampling at a height of 3 cm was achieved using just the top shelter which was raised with 3 screw legs to allow air flow. The bottom of the sampler was covered with a wire mesh and a piece of filter paper was placed directly below the PUF discs to prevent direct contamination with soil particles. The air samplers at the other heights were attached to a post and positioned so they were not placed directly above the sampler immediately below so airflow was not impeded.

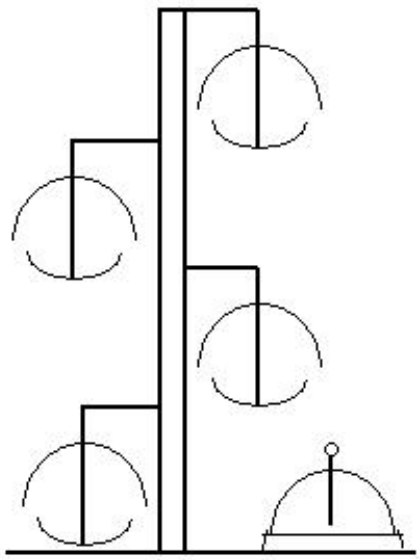


Figure 2. 5: Samplers positioned at graduating heights from the ground

Figure 2. 6: PAS to sample air at 3 cm from the ground

2.2.3 Soil samples

All soil sampling in Birmingham took place at EROS. They were taken from the top 5 cm from 4 different areas within a sectioned off sampling area of 1 m x 1 m adjacent to the air samplers and pooled before being stored in amber glass jars. The soils from different locations in the UK were collected as part of a previous project (Evans, 2007). The Australian soils were collected as part of the National Dioxin Program between 2002 and 2003 (Mueller et al., 2004). The samples were taken from industrial, urban, agricultural and remote locations across Australia. For the purpose of this project 17 samples were analysed from the 86 locations. They were collected from the top 10 cm using aluminium tubes from 3 subsampling sites which were combined to form a composite sample. This was sealed in aluminium foil and freeze dried prior to storage. Additional samples were taken from a suburban garden in West London where there was found to be elevated levels of HBCDs. Samples were taken on 4th April 2010 at increasing distances from the house at approximately 3, 5, 7 and 12 metres in a 14 metre length garden. Soils were stored in amber glass jars at 4^{o}C to prevent photolytic and thermal degradation.

2.2.4 Grass samples

All grass samples were collected from the same 1 m x 1 m area as the soil samples adjacent to the air samplers. A study at the same site identified the grass be a mixed sward comprising of predominantly Perennial ryegrass (*Lolium perenne*), Yorkshire Fog (*Holcus lanatus*), Creeping soft-grass (*Holcus mollis*), Common bent (*Agrostis capillaris*) and three non-grass herbs (*Cerastium fontanum, Stellaria graminea and Plantago lanceolata*) (Currado 1999). These species are typical of those found in UK pasture grasslands (Currado 1999, Thomas et al., 1998). The grass was cut back within this 1 m^2 area and then fresh grass growth was collected approximately every two weeks. The grass was rinsed carefully with distilled water to remove any adhering soil particles, before being freeze dried, homogenised using a grinder and stored in amber glass jars at 4 ^oC prior to analysis.

2.2.5 Curtain samples

Curtain samples which had been stored in the presence and absence of light were provided by National Institute for Environmental Studies in Japan in order to determine the HBCD degradation products. Three black textiles (95-99% polyester) and 3 light blue textiles (polyester) were used. For each type of textile one was shielded from light, one was exposed to light over 371 days, and one sample was covered in aluminium foil and also left in sunlight for 371 days as a dark control sample (Kajiwara and Takigami, 2010). The samples were stored wrapped in foil and kept at 4 ^oC prior to analysis.

2.3 Sample extraction and clean up

2.3.1 Sample extraction and clean up for HBCD

2.3.1.1 Air

Air samples comprising one or two PUF discs were spiked with 10 ng of ^{13}C-labelled α-, β- and γ-HBCD in hexane, and extracted with hexane:DCM (50:50, v/v) in a soxhlet apparatus for 8 hours. The sample was cleaned by passing through a column containing 8 g of acid silica and eluted with 30 mL hexane:DCM (50:50, v/v). The sample was solvent exchanged into 200 μL methanol containing 5 ng d_{18}-γ-HBCD as a recovery determination standard.

2.3.1.2 Soil

Approximately 50 g of soil was accurately weighed into a clean glass beaker and mixed with 50 g of pre-extracted anhydrous sodium sulfate and 5 g copper powder. More sodium sulfate was added if the sample was particularly wet. The soil was then transferred to a pre-cleaned soxhlet thimble (Whatman 41 mm id, 123 mm length) which was then placed in the soxhlet apparatus and spiked with 10 ng of ^{13}C-labelled HBCDs. The samples were then extracted with acetone:hexane (60:40, v/v) in soxhlet apparatus for 8 hours. The acetone was removed by shaking with 2 x 50 mL of distilled water, the lower aqueous phase was discarded to waste and the hexane layer retained.

For the Australian soils approximately 100 g was extracted using ASE (ASE 300, Dionex). The soil was transferred to an ASE cell and treated with 10 ng of ^{13}C labelled α-, β- and γ-HBCD internal standard. The ASE conditions were as follows: temperature 50 ^{o}C, pressure 1500 psi, heat time 5 minutes, static time 5 minutes, flush volume 50 %, purge time 60

seconds, static cycles 3, the solvent used was hexane:DCM (40:60, v/v). The samples were cleaned up using the same method as applied to UK soil samples.

All extracts were reduced using a Turbovap sample concentrator to approximately 0.5 mL, prior to transfer to a pre-cleaned column containing 50 g of acid silica topped with 1 g sodium sulfate and 3 g of copper powder and eluted with 100 mL hexane:DCM (50:50, v/v). The eluate was concentrated in a Turbovap tube to 0.5 mL in hexane, and transferred to a finger vial with washes of 3 x 0.5 mL of hexane and 2 mL of sulfuric acid added. This was mixed well and allowed to separate for at least 2 hours in a cold room stored at 4 ^{0}C. The acid layer was then carefully removed and an additional 2 mL of sulfuric acid was added and mixed well. They were then again left to separate fully, stored in a cold room at 4 ^{0}C. The extract was then passed through a florisil column containing 1.5 g of florisil topped with sodium sulfate and eluted with 30 mL hexane:DCM (50:50, v/v). The sample was solvent exchanged into 200 µL methanol containing 5 ng d_{18}-γ-HBCD as a recovery determination standard.

2.3.1.3 Grass

Approximately 6 g of freeze dried grass was extracted using identical ASE conditions as applied to the Australian soil samples and cleaned up for analysis in the same way.

2.3.1.4 Curtain samples

Curtain samples were also analysed for HBCD degradation products. They were extracted with 20 mL hexane:DCM (50:50, v/v) using ultrasonication for 20 minutes before being prepared for analysis as specified for the air samples in section 2.3.1.1 and analysed using LC/MS-MS according to section 2.4.1.

2.3.2 Sample extraction and clean up for PCBs

2.3.2.1 Air

The PUF discs were transferred with solvent cleaned tweezers and the foil they were stored in rinsed with hexane into the soxhlet apparatus. A known amount of internal standard comprising of PCBs 34, 62, 119, 131 and 173 (10 ng) was added to the sample and it was extracted by soxhlet for 8 hours with hexane. The extract was concentrated under nitrogen using a turbovap to approximately 0.5 mL. This concentrate was transferred to a finger vial and the turbovap tube rinsed with 3 x 0.5 mL hexane and transferred to the vial so the sample was approximately 2 mL. The sample was then mixed with 2 mL concentrated sulfuric acid on a whirlimixer and allowed to separate overnight in a cold room at 4 ^{0}C in the dark.

The top hexane layer was transferred to a clean 100 mL separating funnel along with rinses of the finger vial with 3 x 1 mL aliquots of hexane. Once in the separating funnel, 10 mL of DMSO was added. The funnel was shaken for 2 minutes before being left to separate. The bottom DMSO layer was transferred to a second clean 100 mL separating funnel. This step was repeated twice more with 10 mL DMSO resulting in the sample being extracted in a total of 30 mL DMSO. The first separating funnel and the hexane layer were then discarded. The sample in DMSO was then combined with 40 mL DDW, 30 mL hexane and approximately 0.5 g sodium chloride (NaCl, Fisher Chemicals, Reagent grade), to break down the DMSO-PCB complex. The funnel was shaken for 2 minutes and allowed to separate into two layers with hexane at the top and DMSO/H_2O at the bottom. The bottom DMSO/H_2O layer was transferred to a clean 100 mL glass beaker and the hexane layer was then transferred to a Turbovap tube. The DMSO/H_2O layer was then transferred back in the separating funnel and another 30 mL of hexane was added and the process repeated. This step was repeated one further time before the DMSO layer was discarded.

The extract in hexane was concentrated under nitrogen in the turbovap tube to approximately 0.5 mL before being cleaned on a precleaned florisil column containing 1 g of florisil column topped with 1 g sodium sulfate. It was eluted with 20 mL hexane, reduced under nitrogen and transferred to a vial insert with two washes of the vial with hexane. The sample was finally reduced to near dryness before 50 uL recovery determination standard (PCBs 19 and 129) in nonane was added.

2.3.2.2 Soil

Approximately 30 g of soil was accurately weighed and mixed with pre-extracted hydromatrix. This was transferred to a 66 mL ASE cell and treated with 10 ng of internal standard containing PCBs 34, 62, 119, 131 and 173. This was then extracted with hexane using accelerated solvent extraction (ASE 300, Dionex). The conditions were: temperature 150 ^{o}C, pressure 1500 psi, heat time 7 minutes, static time 5 minutes, flush volume 50%, purge time 100 seconds, static cycles 3.

The soil extract was then cleaned using the same method for air except the final florisil column was topped with 1 g $AgNO_3$-impregnated aluminium oxide to remove sulfur. A large amount of sulfur was sometimes found in the soil samples, therefore they required an additional clean up with silver impregnated aluminium oxide. For all soil samples, the final florisil column was topped with 1 g of silver impregnated aluminium oxide.

The preparation was done in dark room conditions as the mixture degrades quickly from exposure to light. Because of the nature of the mixture to degrade quickly it was made just prior to use and only used on the day it was prepared. Into a foil covered 50 mL conical flask 0.75 g of $AgNO_3$ (Fisher chemicals ultra pure grade) was dissolved in 0.75 mL of accurately

weighed DDW along with 10 g of pre-extracted aluminium oxide. The DDW was added using a Gilson pipette pre-calibrated on the same balance. The mixture was shaken in a shaker apparatus for 45 minutes followed by a further 2 minutes shaking by hand. The mixture was added to the florisil column which was completely wrapped in aluminium foil to prevent degradation.

2.3.2.3.Grass

Approximately 8 g of freeze dried grass was transferred to a 66 mL ASE cell, treated with 10 ng of internal standard and extracted using the same conditions used for soil samples. This was followed by elution through an acid silica column. Approximately 20 g of acid silica was precleaned with hexane. The extract was concentrated to approximately 0.5 mL, added to the column and eluted with 50 mL hexane collected in a clean turbovap tube. The extract was then reduced to approximately 1 mL and transferred to a 100 mL separating flask to be purified by DMSO extraction followed by a florisil column as specified for air and soil samples.

2.4 Analysis

2.4.1 LC/MS/MS analysis of HBCDs

2.4.1.1 Determination of HBCD diastereoisomers

Individual HBCD diastereoisomers were separated and analysed using LC-MS/MS. The equipment used was a Shimadzu LC-20AB Prominence liquid chromatograph interfaced with a Sciex API 2000 triple quadrupole mass spectrometer. The diastereoisomers were separated using a C18 reversed phase analytical column (150 mm x 2 mm i.d., 3 μm particle size). The mobile phases used were (a) 1:1 methanol/water and (b) methanol at a flow rate of 150 μL

min^{-1}. The elution program was started at 50% (b) then increased linearly to 100% (b) over 7 min, held for 4 min followed by a linear decrease to 60% (b) over 4 min, held for 1 min and ending with 100% (a) for 10 min. The HBCD isomers were monitored using m/z 640.6→79, m/z 652.4→79 and m/z 657.7→79 for the native, ^{13}C-labelled and d_{18}-labelled isomers respectively.

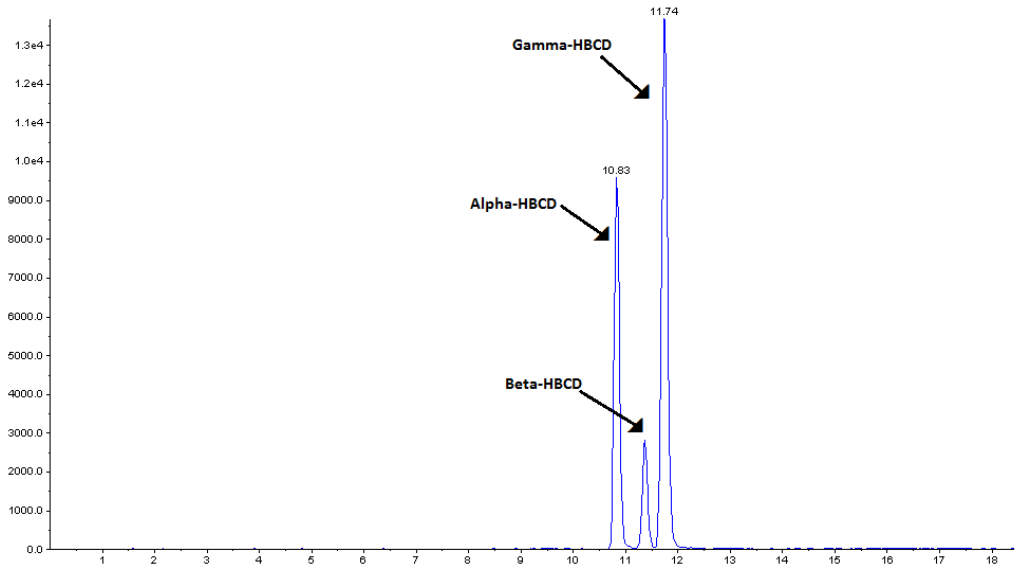


Figure 2.7: Chromatogram showing the separation of the native HBCD diastereoisomers in soil sample from Lancaster, UK

Table 2. 1 MS/MS parameters for the determination of HBCD diastereoisomers

Parameter	Value (units)
Curtain gas	35 (a.u.)
Turbo gas temperature	500 (^{o}C)
Ion spray voltage	-4500 (V)
Declustering potential	-5 (V)
Focusing potential	-365 (V)
Collision gas	5 (a.u.)
Collision energy	40 (eV)
Cell entrance potential	-6 (V)
Collision cell exit potential	-10 (V)

2.4.1.2 Determination of HBCD degradation products PCBD and TBCD

Two classes of degradation products of HBCD, the pentabromocyclododecenes (PBCDs) and tetrabromocyclododecadienes (TBCDs) were monitored at transitions *m/z* 560.8→79 and *m/z* 480.4→79, respectively using the same column and conditions as for the HBCD diastereoisomers. These degradation products could not be accurately quantified as there is not as yet reference standards available with which response factors may be calculated. Their concentrations were therefore calculated in a semi-quantifiable fashion using the average relative response factor for the three HBCD diastereoisomers. PBCD peaks 3 and 4 were not fully resolved; however alterations in the solvent gradient and flow rate will have affected the separation of α-, β- and γ-HBCD.

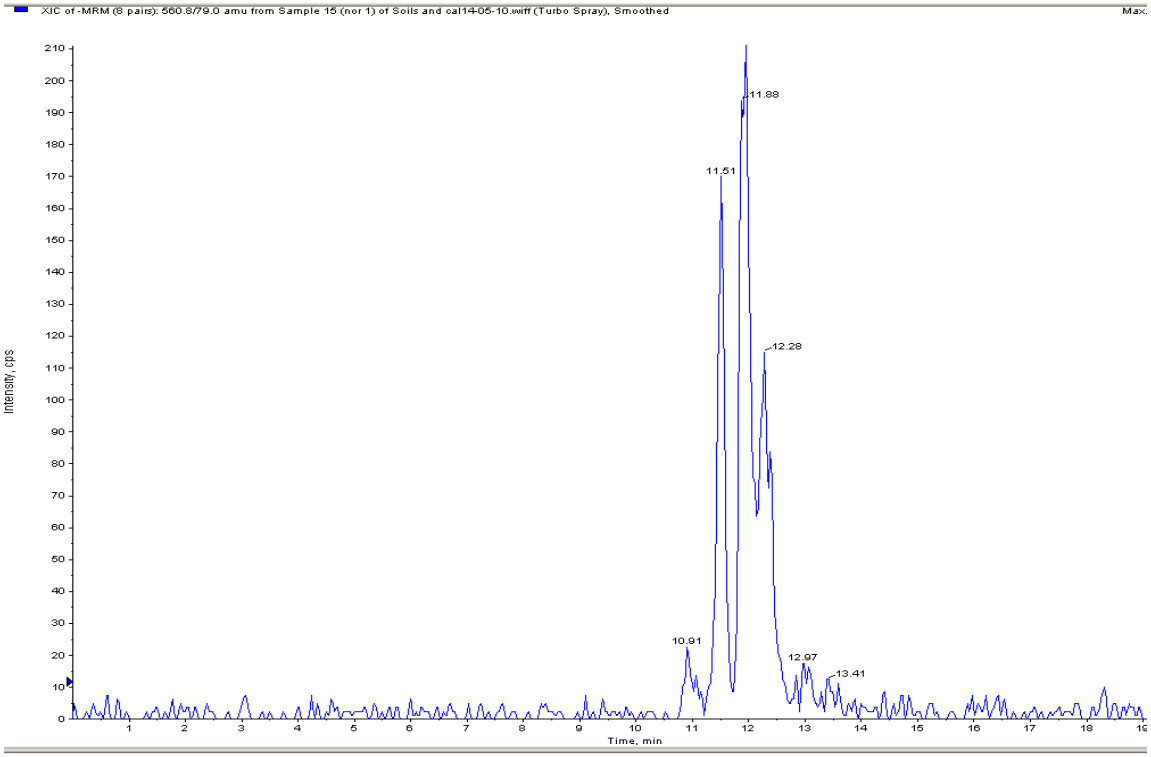


Figure 2.8: PBCDs in a soil sample

2.4.1.3 Determination of chiral signatures of HBCDs

The HBCD enantiomers were separated using a chiral permethylated cyclodextrin LC column (200 mm x 4 mm I.D., 5 μm particle size) (NUCLEODEX beta-PM, Macherey-Nagel; GmbH & Co, Düren, Germany). The separation used mobile phases of: a) 1:1 methanol/water with 2 mM ammonium acetate and (b) 3:7 methanol/acetonitrile at a flow rate of 500 μL/min. Starting with 50 % (b) it then increased linearly to 100 % (b) over 4.5 min and held for 5.5 min, followed by a linear decrease to 65 % (b) over 4 min and then held for 2 min.

There is potential for matrix effects which can result in incorrect EFs being calculated Therefore the EFs are corrected according to the labelled internal standard as detailed by Marvin et al. (Marvin et al., 2007). The following equation is used:

$$EF_{corrected} = \frac{\left[\left(A^{+} / A^{+}{}_{labelled}\right) \times \left(pgA^{+}{}_{labelled}\right)\right]}{\left[\left(A^{+} / A^{+}{}_{labelled}\right) \times \left(pgA^{+}{}_{labelled}\right)\right] + \left[\left(A^{-} / A^{-}{}_{labelled}\right) \times \left(pgA^{-}{}_{labelled}\right)\right]} \quad \text{(Equation 2.1)}$$

Where A^{+} and A^{+} are the peak areas of the (+) enantiomer and (-) enantiomer respectively, $A^{+}_{labelled}$ $A^{-}_{labelled}$ are the peak areas of the labelled (+) enantiomer and (-) enantiomers and $pgA^{+}_{labelled}$ and $pgA^{-}_{labelled}$ are the masses of the labelled isomers in picograms.

2.4.2 GC/MS analysis of PCBs

2.4.2.1 Determination of native PCBs

PCB analyses were conducted on an Agilent 5975C GC/MS system fitted with a 30 m HP5-MS column (0.25 mm id, 0.25 μm film thickness). Both injector and interface temperatures were 280 ˚C. The oven temperature program was: 140 ˚C for 2 min, 5 ˚C/min to 215 ˚C and

held for 5 min, then 2˚C/min to 280 ˚C. The mass spectrometer was operated in EI+ SIM mode. The PCBs analysed were PCB 28+31, 52, 101, 118, 153, 138 and 180 and the total PCB concentration represents the sum of these congeners.

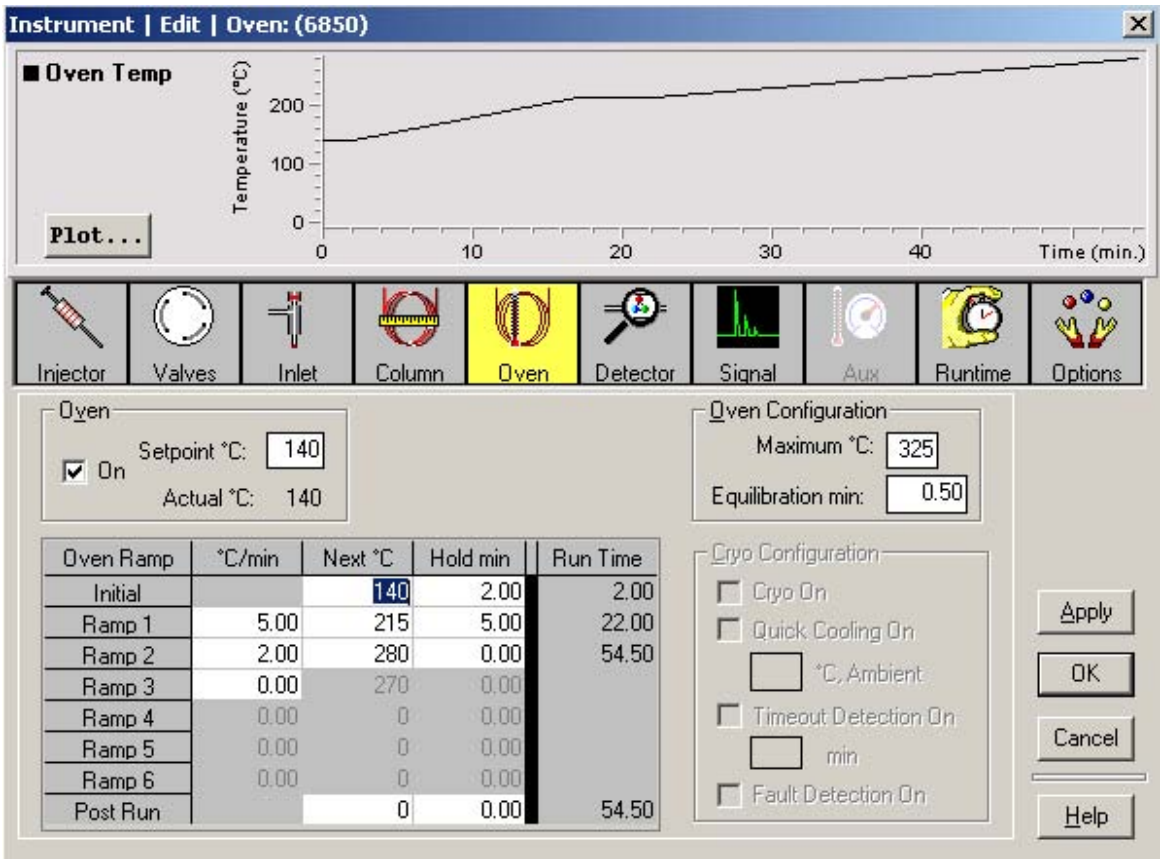


Figure 2.9: Details of the GC temperature programme for separation of PCBs

Table 2.2 Ions monitored for PCBs

Compound	Molecular weight	Ions			
		Quantification ion		Secondary ion	
Tri	257.5	255.95	$(M)^+$	257.95	$(M+2)^+$
Tetra	292	289.95	$(M)^+$	291.95	$(M+2)^+$
Penta	326.5	325.9	$(M)^+$	327.90	$(M+2)^+$
Hexa	361	359.9	$(M)^+$	361.90	$(M+2)^+$
Hepta	395.5	393.9	$(M+2)^+$	395.85	$(M+4)^+$

2.4.2.2 Determination of Enantiomer fractions

PCBs enantiomer fractions were determined on the same instrument (Agilent 5975C GC/MS) using a ChiraSil-Dex column (25 m x 0.25 mm x 0.25 μm film thickness). The temperature program began at 140 ˚C held for 1 minute then 30 ˚C min^{-1} to 160 ˚C and held for 20 minutes, then 1 ˚C min^{-1} to 170 ˚C held for 20 minutes and finally 1 ˚C min^{-1} to 180 C and held for 40 minutes. One μL of sample was injected for analysis.

Table 2.3 Chiral PCBs

Compound	Molecular weight	Ions	
		Quantification ion	Secondary ion
PCB 95	326.5	325.9	327.9
PCB 136	361	359.9	361.9

2.5 Validation of analytical protocols

2.5.1 RRFs

A 5 point calibration was undertaken before the samples were run. This was used to calculate the RRF (relative response factor) for each of the target analytes.

The RRF was calculated by the equation

$$RRF = \frac{A_{NAT}}{A_{IS}} x \frac{C_{IS}}{C_{NAT}} \qquad \text{(Equation 2.2)}$$

Where A_{NAT} is the native standard peak area, A_{IS} is the internal standard peak area, C_{NAT} is the concentration of the native standard and C_{IS} is the concentration of the internal standard.

2.5.2 Internal standard (IS), recovery determination standard (RDS) and sampling evaluation standard (SES)

For PCBs the internal standard was made up of five native PCB congeners which are not found at significant levels in the environment (Ayris et al, 1997). These were 34 (tri), 62 (tetra), 119 (penta) 131 (hexa) and 173 (hepta). Each congener was used to quantify its corresponding homologue group. Therefore PCB 34 was used to quantify trichlorobiphenyls, PCB 62 was used for tetrachlorobiphenyls and so on.

For the HBCDs the native compounds were determined in the samples by the addition of isotopically labelled internal standards, where the carbon atoms were ^{13}C. The labelled compounds have nearly identical physical and chemical properties but are monitored at a different mass. It therefore accounts for any losses during the extraction and cleanup process. The internal standard used to quantify the HBCD diastereoisomers were ^{13}C labelled α, β and γ-HBCDs.

Table 2.4 Standards used in samples for PCB analysis

PCB congeners							
19	129	34	62	119	131	173	147
RDS		IS					SES

Table 2.5 Standards used in samples for HBCD analysis

HBCD standards				
d_{18}-γ-HBCD	^{13}C-α-HBCD	^{13}C-β-HBCD	^{13}C- γ HBCD	d_{18}-α-HBCD
RDS	IS			SES

The relative response factors (RRF) was calculated from calibration standards run at the beginning and end of each run. This was used to calculate concentrations of the native compounds in the sample.

A recovery determination standard (RDS) was added to the samples at the final stage of analysis and was used to calculate the percentage recovery of the internal standard. For HBCD this was d_{18}-γ-HBCD and for PCBs, PCB 19 was used for the tri and tetra PCBs and PCB 129 for the penta, hexa and hepta PCBs. If recoveries of the IS were below 30% then the data was acceptable only if the signal to noise ratio of the internal standard exceeded 20:1 (Ambidge et al, 1990). The internal standard recovery was calculated using the equation:

$$\%ISrecovery = \left[\left(\frac{A_{IS}}{A_{RDS}} \right)_S \times \left(\frac{A_{RDS}}{A_{IS}} \right)_{STD} \times \left(\frac{C_{IS}}{C_{RDS}} \right)_{STD} \times \left(\frac{C_{RDS}}{C_{IS}} \right)_S \right] \times 100 \quad \text{(Equation 2.3)}$$

Where $(A_{IS}/A_{RDS})_S$ is ratio of peak areas of the IS to the RDS in the sample and $(A_{RDS}/A_{IS})_{STD}$ is the ratio of peak areas of the RDS to the IS in the calibration standard, $(C_{IS}/C_{RDS})_{STD}$ is the ratio of the concentration of the IS and RDS in the calibration standard and $(C_{RDS}/C_{IS})_S$ is the concentration of the RDS and IS in the sample assuming 100% recovery of the IS.

For air samples only, a sampling evaluation standard (SES) was added before to the PUF discs in the PAS they were deployed at the sampling site. For HBCDs d_{18}-α-HBCD was used while PCB 147 was used as an SES for the PCBs. Recoveries of the SES were calculated as in equation 2.4.

$$SES\%recovery = \left[\left(\frac{A_{SES}}{A_{RDS}} \right)_S \times \left(\frac{A_{RDS}}{A_{SES}} \right)_{STD} \times \left(\frac{C_{SES}}{C_{RDS}} \right)_{STD} \times \left(\frac{C_{RDS}}{C_{SES}} \right)_S \right] \times 100 \quad \text{(Equation 2.4)}$$

Where $(A_{SES}/A_{RDS})_S$ is ratio of peak areas of the SES to the RDS in the sample and $(A_{RDS}/A_{SES})_{STD}$ is the ratio of peak areas of the RDS to the SES in the calibration standard, $(C_{SES}/C_{RDS})_{STD}$ is the ratio of the concentration of the SES and RDS in the calibration standard and $(C_{RDS}/C_{SES})_S$ is the concentration of the RDS and SES in the sample assuming 100% recovery of the SES.

Table 2.6 PCB IS and SES recoveries (%)

Congener	Min	Max	Mean	Std dev	RSD %
AIR (n=56)					
34	21	76	50	13	26
62	24	82	56	13	22
119	29	86	66	13	20
131	32	89	70	14	19
173	34	97	74	15	20
147 (SES)	29	99	67	18	26
SOIL (n=14)					
34	24	74	51	11	22
62	31	66	52	9	16
119	35	66	58	8	15
131	42	72	62	8	13
173	45	89	69	12	17
GRASS (n=14)					
34	41	68	54	8	14
62	40	69	56	8	15
119	52	77	67	7	10
131	58	79	71	6	8
173	57	88	75	9	12

Table 2.7 HBCD IS and SES recoveries (%)

HBCD	Min	Max	Mean	Std dev	%RSD
AIR					
α-HBCD	37	83	59	14	24
β-HBCD	30	82	50	16	33
γ-HBCD	46	109	73	19	26
SES	30	71	50	12	25
SOIL (UK)					
α-HBCD	22	142	79	35	44
β-HBCD	21	150	59	28	48
γ-HBCD	20	79	46	18	39
SOIL (Aus)					
α-HBCD	38	152	90	34	38
β-HBCD	29	121	63	27	43
γ-HBCD	30	153	93	34	36
GRASS					
α-HBCD	52	106	70	26	37
β-HBCD	18	31	26	6	25
γ-HBCD	41	82	58	18	30

2.5.3 Concentrations in samples

Concentrations in samples were calculated using the following equation:

$$Concentration = \frac{A_{NAT}}{A_{IS}} \times \frac{1}{RRF} \times \frac{M_{IS}}{SS} \qquad \text{(Equation 2.5)}$$

HBCDs and PCBs were only quantified provided the following criteria were met:

1. The signal to noise ratio (S/N) exceeded 3:1
2. The relative retention time (RRT) of the peak in the sample was within ± 0.2% of the average value determined for same congener in the 2 calibration standards run for that sample batch.
3. The chlorine/bromine isotope ratios were within ± 20% of the average for the 2 calibration standards run for that sample batch.

2.5.4 Blanks and Limit of detection (LOD)

One blank sample was run approximately every 6th sample. For air samples this consisted of a two pre extracted PUF disks taken to the sampling site in a sampler housing but not deployed and for soil and grass samples this was a reagent blank. They were extracted and cleaned up as described for the samples. Blanks were acceptable when the concentrations were less than 5% of that measured in the samples and the samples were corrected when the concentrations fell between 5-20% of the lowest concentration in the batch. Results were not reported for any batch with blanks exceeding 20% the lowest concentration in the batch.

PCB concentrations in the blanks were less than 5% of the concentrations detected in the samples so were not corrected. PCBs 95 and 136 were not detected in any of the blanks. For the HBCD UK soil samples there was correction as low levels of HBCD were detected in the blanks of between 0.1 and 0.4 ng/g ∑HBCD. HBCD was below the limit of detection in the blanks for the Australian soils.

The limit of detection (LOD) was defined as the amount of contaminant that gives a signal to noise ratio of 3:1. When the concentration of the blank exceeded the LOD then the blank concentration was used as the LOD. For the purposes of calculating descriptive statistics half the LOD was used when the compounds were not detected. The on column LODs for HBCD α-, β- and γ-HBCD were 1.3, 0.7 and 1.7 pg respectively. The sample limit of quantification (LOQ) is the lowest measurable concentration in the sample and was calculated according to the following equation

$$LOQ = \frac{LOD \times FEV}{VFEI \times SS} \times \frac{100}{\%IS\,\mathrm{Recovery}} \qquad \text{(Equation 2.6)}$$

Where FEV is the final extract volume (µl), VFEI is the volume of final extract injected (µl) and SS is the sample size. For HBCDs the LOQs were governed by the reagent blanks for the UK soils where assuming 50 g sample size was 30 pg/g. The LOQ for grass and air samples the LOQs were 8.8pg/g and 2.0 pg/m^3 ∑HBCD respectively. For the Australian soils the LOQ was calculated to be 0.5 pg/g ∑HBCD. The LODs and LOQs of PCBs are shown in table 2.8.

Table 2.8 Limits of detection and limits of quantification of PCBS in air, soil and grass

Congener	LOD (pg)	LOQ		
		AIR (pg/m^3)	SOIL (pg/g)	GRASS (pg/g)
PCB 28+31	0.23	0.42	0.75	2.7
PCB 52	0.09	0.15	0.29	1.0
PCB 101	0.55	0.76	1.6	5.1
PCB 118	0.59	0.81	1.7	5.5
PCB 153	0.30	0.39	0.81	2.6
PCB 138	0.37	0.48	0.99	3.3
PCB 180	0.13	0.16	0.31	1.1

2.5.5 Accuracy and precision

Accuracy and precision was determined by the analysis of a standard reference material (SRM). PCBs were determined in 5 replicate analyses of SRM 2585. The relative standard deviations (RSDs) obtained ranged between 4-11% showing good precision of the method.

Table 2.9 Concentrations of PCBs (ng/g) in NIST SRM 2585 compared to the certified values

Congener	1	2	3	4	5	Mean	σ_{n-1}	RSD%	Certified Conc.	% deviation from certified value
PCB 28+31	26.6	27.7	32.0	31.9	31.1	29.9	2.6	9	27.4±0.5	9.0
PCB 52	21.2	20.3	20.3	22.4	22.8	21.4	1.1	5	21.8±1.9	-1.9
PCB 101	32.9	30.1	27.8	29.3	29.9	30.0	1.8	6	29.8±2.3	0.7
PCB 118	31.9	29.6	29.2	32.8	32.5	31.2	1.7	6	26.3±1.7	18.6
PCB 153	40.2	37.4	37.9	40.7	40.0	39.2	1.5	4	40.2±1.8	-2.4
PCB 138	34.6	35.7	28.2	38.1	36.5	34.6	3.8	11	27.6±2.1	25.5
PCB 180	21.2	17.6	19.6	19.8	21.3	19.9	1.5	8	18.4±3.2	8.1

There was not an available standard reference material for HBCDs at the time of the analysis therefore HBCDs were determined in replicate analysis of SRM 2585 house dust where values have been reported previously (Keller et al., 2007). The results in table 2.10 show there was good precision of the method with RSDs of between 6 and 15%.

Table 2.10 Concentrations of HBCDs in NIST SRM 2585 (ng/g) compared to indicative values (Keller et al., 2007)

	1	2	3	4	5	Mean	σ_{n-1}	RSD %	Indicative Conc.
α-HBCD	18.6	19.2	21.7	22.3	19.3	20.2	1.7	8	19±3.7
β-HBCD	5.0	4.3	4.7	5.8	6.2	5.2	0.8	15	4.3±1.1
γ-HBCD	126.5	138.6	130.9	126.5	117.3	128.0	7.7	6	120±22

2.5.6 Passive air sampling rates

Passive samplers need to be calibrated in order to determine the rate at which they sample air and therefore convert the masses of target analytes detected in the PUF discs to atmospheric concentrations. This can be determined by a calibration experiment using low volume active sampling alongside passive samplers. For PCBs the passive sampling rates were taken from the outdoor sampling rates from a previous study and are shown in table 2.11 (Evans, 2007).

Table 2.11 Calculated outdoor passive sampling rates (m^3 day^{-1}) for PCBs (Evans, 2007)

	Average sampling rate
TriCB	3.95
TetraCB	3.99
PentaCB	4.33
HexaCB	4.53
HeptaCB	5.17

For HBCDs a calibration experiment was undertaken for outdoor air. A low volume active air sampler (Capex L2X, Charles Austin, UK) operated at a flow rate of 4 L min^{-1} was set up at the EROS site and run over a period of 50 days to collect approximately 288 m^3 of air. The sampler was attached to a precleaned glass tube (3 cm diameter, 25 cm in length) containing two PUF plugs to collect the gaseous phase. The tube was covered in aluminium foil to prevent exposure to sunlight and therefore prevent photodegradation of the HBCDs. This was attached with plastic tubing to a filter holder containing a 47 mm diameter membrane filter (1.0 μm pore size Whatman UK) to collect the particulate phase. The PUFs and filters were changed every 10 days to minimise analyte breakthrough and combined at the end to make one sample. A Gilian gilibrator was used to calibrate the sampler at the beginning and end of the experiment.

Simultaneously PUF samplers were set up as described previously and deployed at EROS (n=9) over 50 days and collected at 10 day periods. To ensure detectable amounts of HBCDs, 4 and 2 PUF samplers were collected and combined for 10 and 20 days respectively.

To determine the sampling rates of each compound, the equivalent air volumes sampled by each PUF disk over a given exposure period, V_{eq} (cm^3), were calculated using the following equation

$$V_{eq} = \frac{M}{C_A} = k_A \times A_{PUF} \times \Delta t \qquad \text{(Equation 2.7)}$$

Where M is the mass of the compound collected in the PUF disc, C_A is the concentration (pg cm^{-3}) of the target analyte determined from the active air sampler, k_A is the air side mass transfer velocity (cm sec^{-1}), A_{PUF} is the exposed macro surface area of the PUF disk (cm^2), and Δt is the sampling period (seconds).

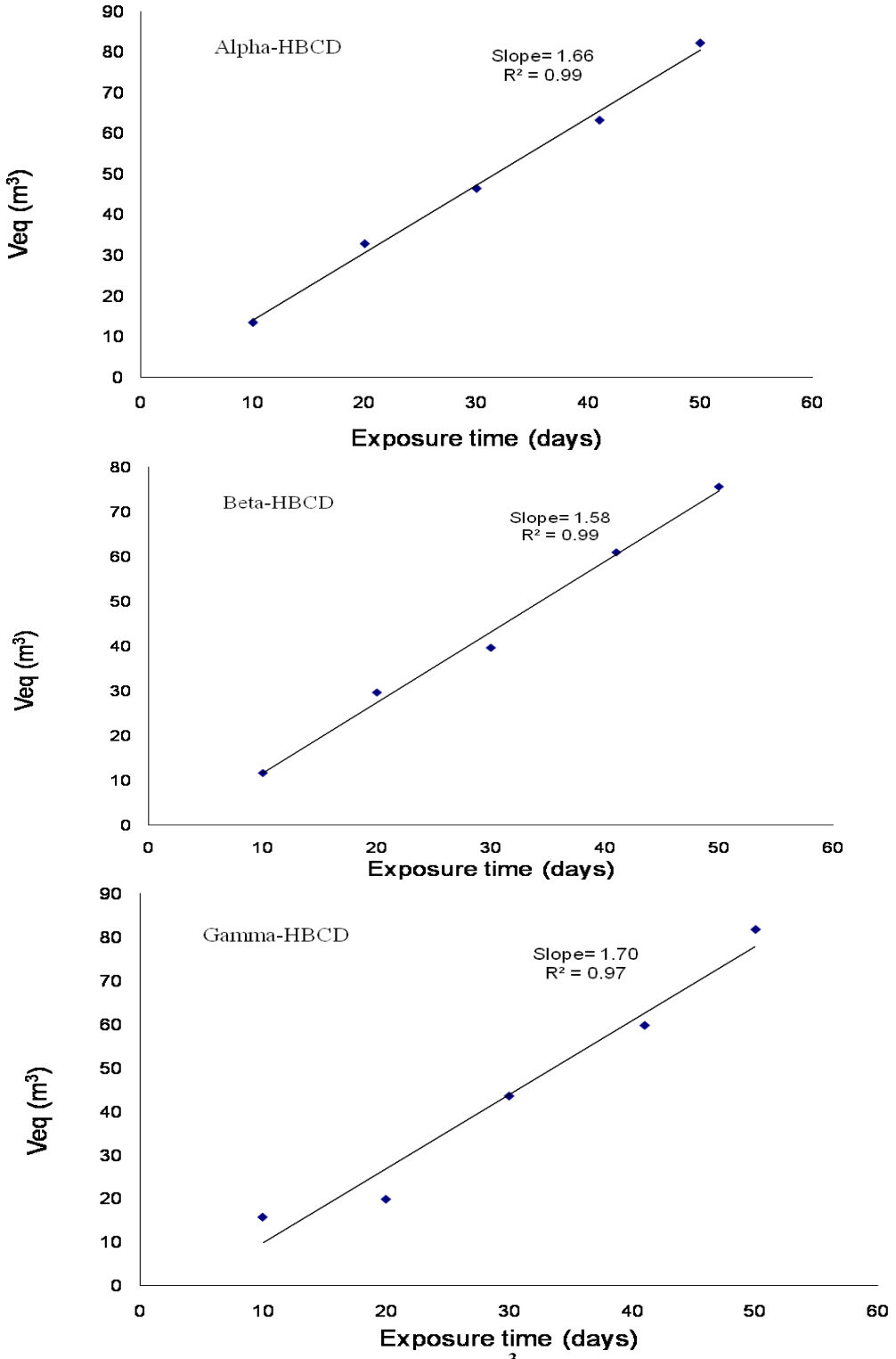


Figure 2.10 Equivalent air volume (Veq, m^3) versus exposure time (days) for α, β, and γ-HBCD where the slope is equal to the passive air sampling rate (m^3 day^{-1}).

Table 2.12 Masses of HBCD diastereoisomers detected in air samples collected during calibration experiment (pg).

HBCD diastereoisomer/sampling interval (days)	10	20	30	41	50
α-HBCD	65	164	232	316	411
β-HBCD	32	89	119	183	227
γ-HBCD	126	179	392	538	736

The sampling rates were calculated to be 1.66, 1.58 and 1.70 m^3 day^{-1} for α, β, and γ-HBCD respectively. These results are higher than the sampling rates for indoor air calculated by Harrad and Abdallah, of 0.87, 0.89 and 0.91 m^3 day^{-1} for α, β, and γ- HBCD respectively for samplers with the same fully sheltered configuration (Harrad and Abdallah, 2008). This variation is likely to be due to the sampling taking place outdoors rather than indoors reflecting the greater exposure to air of samplers deployed outdoors as a result of the higher wind speeds.

2.6 Statistical Analysis

The software used for statistical analysis was Excel (Microsoft 2007) for calculation of descriptive statistics and t-tests. Additionally, SPSS version 19 was used to determine that data distribution was normal using both Kolmogorov-Smirnov and Shapiro-Wilk tests, and consequently to conduct the repeated measures ANOVA test in Chapter 3. The significance level assumed was 0.05. When the concentration of the compound was below the LOD, the concentrations was assumed to equal half the LOD for the purpose of statistical testing.

CHAPTER 3: CONCENTRATIONS AND CHIRAL SIGNATURES OF PCBs IN SOIL, AIR AND GRASS FROM BIRMINGHAM UK

3.1 Synopsis

This chapter employs measurements of chiral signatures of PCBs 95 and 136 in samples of soil, grass and air on a vertical transect to test the hypothesis that PCBs in grass occur principally via vapour phase foliar uptake of PCBs that have volatilised from soil (Harrad et al., 2006). This chapter also builds on previous work that examined chiral signatures of PCBs in outdoor air and soil and found chiral signatures of PCB 95 to display appreciable deviation in soil which was not seen in air samples (Robson and Harrad, 2004, Jamshidi et al., 2007).

3.2 Sampling Strategy

Five passive air samplers (PAS) were deployed at graduating heights from the ground at a single site on the University of Birmingham campus. Samples were collected fortnightly over a four month period in 2009 (starting on 3rd June) and a three month period in 2010 (starting on 26th March). The samplers were positioned at heights of 3, 10, 40, 90, and 130 cm above the soil surface. The four highest samplers were a fully-sheltered design whereas the 3 cm lowest sampler was not fitted with the bottom stainless steel shelter in order to sample the air at the air:soil interface. The sampling rates for the top 4 samplers were 3.95, 3.99, 4.33, 4.53, and 5.17 m3 day^{-1} for tri-, tetra-, penta-, hexa-, and heptachlorinated biphenyls, respectively (Evans, 2007). Air sampling rates for the specially-adapted sampler used at the 3 cm height could not be determined as linear uptake of PCBs over a calibration exercise was not observed with the sampler in this configuration. However, it was still possible to determine the masses

and thus the congener pattern of PCBs present in air samples taken at this height. Air sampling rates are assumed to be non-enantioselective so it was possible to compare EFs of PCB 95 and 136 in air samples taken at each of the 5 heights studied. Low volume active air sampling was undertaken at 3 cm height from the ground to determine the concentrations.

Samples of grass and soil were collected from a 1 m x 1 m plot located adjacent to the PAS at the end of each air sampling period as detailed in sectioned 2.2.3 and 2.2.4. All samples were then analysed for concentrations of PCBs followed by enantioselective analysis for PCBs 95 and 136 as detailed in section 2.4.2.

3.3 Concentrations of PCBs in air, soil and grass

The average concentrations of PCBs found in air, grass and soil during the two sampling campaigns are shown in table 3.1. Table 3.2 shows the concentrations from this study compared to air samples from other studies. The concentrations are higher than those previously seen at the same location in 1999-2000 (74 pg/m^3 ∑PCB) using active sampling and in 2003-2004 (43 pg/m^3 ∑PCB (sum of the same congeners used in this study)) using passive samplers (Harrad and Mao, 2004, Jamshidi et al., 2007). This may be due to the fact that the sampling in this study took place at warmer times of the year when concentrations are higher, whereas the previous studies took place year-round. In the UK the concentrations are less than the average seen in London and fell within the range seen in Stevenage, Cardiff and Manchester (Halsall et al., 1995). The concentrations were higher than those seen for the same congeners in air from Singapore, Japan and South Korea (Jaward et al., 2005).

Tables 3.3 and 3.4 shows the concentrations of soils and grass in this study compared with other studies and sites. The ∑PCB concentrations in soil were found to be 310 pg/g dry

weight in 2009 and 1030 pg/g in 2010. This was slightly lower with those measured at the same site in 2003-2004 which was 1820 ±733 pg/g dw (Jamshidi et al., 2007). The ∑PCB grass concentrations of 1300 pg/g dw in 2009 and 2600 pg/g in 2010 were consistent with that seen at the same site in 1999 of 2388 pg/g dry weight (Currado, 1999) and 1389 pg/g at a German urban site (McLachlan et al., 1995). It was slightly higher than that found in grass from a rural site in Lancaster where the concentration of ΣPCBs were 458 pg/g dry weight (Thomas et al., 1998).

Table 3.1 Average concentrations of ∑PCBs in air (pg/m^3), soil (pg/g dry weight), and grass (pg/g dry weight)

Year/Concentrations		Air (10 cm)	Air (40 cm)	Air (90 cm)	Air (130 cm)	Soil	Grass
2009	Mean	**88**	**150**	**170**	**190**	**310**	**1300**
	σ_{n-1}	6.9	25	33	29	210	420
	Min	76	130	130	160	160	860
	Max	95	200	240	250	810	2100
	5th Percentile	78	129	134	160	162	897
	95th Percentile	95	189	221	233	633	1911
2010	Mean	**60**	**80**	**130**	**150**	**1030**	**2600**
	σ_{n-1}	13	17	26	29	190	1020
	Min	45	55	102	114	780	1200
	Max	78	103	160	180	1200	3700
	5th Percentile	46	58	104	118	799	1342
	95th Percentile	77	100	159	184	1219	3687

Table 3.2 Concentrations of ∑PCBs in air from this and other studies

Sampling site	Average ∑PCB (range) pg/m^3	Reference
This Study 2009 (130 cm)	190	-
This Study 2010 (130 cm)	150	-
EROS, Birmingham, UK (urban)	74	Harrad and Mao, 2004
EROS, Birmingham, UK (urban)	42	Jamshidi et al., 2007
London UK (urban)	1350 (413-3850)[a]	Halsall et al., 1995
Stevenage UK (urban)	370 (141-1840)[a]	
Cardiff UK (urban)	575 (112-1520)[a]	
Manchester UK (urban)	404 (180-844)[a]	
London UK (urban)	1184 (1090-1450)	Coleman et al., 1997
Manchester UK (urban)	400 (340-460)	
Izmir, Turkey (industrial)	1449	Bozlaker et al., 2008
China	7-117	Jaward et al., 2005
Singapore	1.5-14	
Japan	1.6-76	
South Korea	4-29	

Sum of PCBs 28/31, 52, 101, 118, 138, 153 and 180 except [a]Sum of PCBs 28, 52, 77, 101, 118, 138, 153 and 180

Table 3.3 Concentration of ∑PCBs in soil from this and other studies

Sampling site	Average ∑PCB (range) pg/g d.w	Reference
This Study 2009	310	-
This Study 2010	1030	-
EROS, Birmingham, UK (urban)	305	Jamshidi et al., 2007
UK (rural) (n=201)	2537 (63-69717)	Heywood et al., 2006
Canada	11000 (600-51000)[b]	Wong et al., 2009
China	515 (138-1840)[c]	Ren et al., 2007

Sum of PCBs 28/31, 52, 101, 118, 138, 153 and 180 except [b]Sum 28, 52, 101, 118, 138, 153 and 180 and [c]Sum of 51 congeners

Table 3.4 Concentrations of PCBs in grass from this and other studies

Sampling site	Average ∑PCB (range) pg/g d.w	Reference
This Study 2009	1300	-
This Study 2010	2600	-
Birmingham UK	2388	Currado 1999
Lancaster, UK (rural)	458	Thomas et al., 1998
Germany (Urban)	1389[a]	McLachlan, et al., 1995

Sum of PCBs 28/31, 52, 101, 118, 138, 153 and 180 except
[a]Sum of PCBs 28/31, 52, 101, 118, 138 and 153

Figure 3.1: Average ± σ_{n-1} atmospheric concentrations of ∑PCBs at various heights

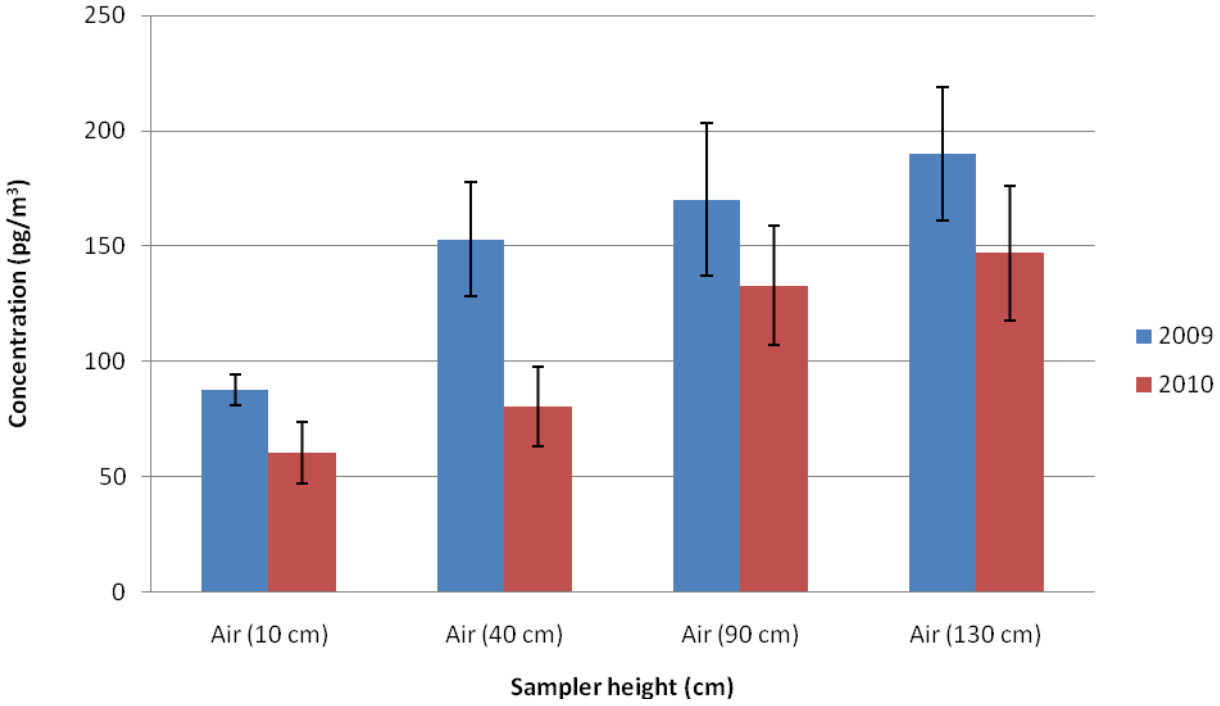


Figure 3.1 shows the concentrations at graduating heights where there was found to be a marked increase in concentration as the sampler got higher from the ground. It is possible that because we used a single air sampling rate for each height of the sampler; any variations in air sampling rate due to height would impact on the concentrations recorded. However active sampling undertaken at 3 cm from the ground (discussed in section 3.6) also showed a lower concentration than that observed in air passively sampled at 10 cm indicating that these results are a true representation of the concentrations present. A repeated measures ANOVA test (SPSS statistics 19) confirmed that there were significant ($p<0.05$) differences between the concentrations at all heights for the 2009 samples. This was also seen with the 2010 samples with the exception of the concentrations at 90 and 130 cm heights. This is different from what was found by Krauss et al., who saw an increase in concentration of PCBs closer to the ground in a study looking at allotment gardens (Krauss et al., 2004). This is likely to be due to a number of differences in the studies. Importantly the ∑PCB concentration of 1.1-634.6 mg/kg (sum of 32 congeners) in this study were considerably higher than ours and so the soil

would exert a much greater influence on air. This study was also undertaken over 13 sites so greater variation is likely to be seen. It has also been seen in the case of OC pesticides (α-HCH, cis- and trans-chlordane, heptachlor (HEPT), o,p'-DDT, heptachlor epoxide (HEPX) and oxychlordane (OXY)) collected at heights ranging from 5-140 cm from the surface that concentration declined with height (Finizio et al., 1998). This was also from a contaminated site (concentrations ranging from 42 to 2496 ng/g dw) so the soil had a greater effect on air than seen in our study. Our results are similar to values recorded at the same site in 2005-2006 where there was a small increase in concentration from five samples taken from 10, 25, 40, 55 and 70 cm from the ground (Evans, 2007). This study found that ∑PCBs measured 244 pg/m^3 (sum of 84 PCB congeners) at 10 cm and gradually increased to 360 pg/m^3 at 70 cm with lowest variation between the two highest samplers. It was also observed in our study that the soil concentration was over 3 times higher in 2010 (1030 pg/g ∑PCB) compared to 2009 (310 pg/g ∑PCB) and yet the air concentration was found to be lower in 2010 implying that the soil concentration is not contributing to concentrations in air sampled at 10 cm and above. Our finding of higher concentrations furthest from the soil surface suggests that at the background soil concentrations observed in this study; volatilisation from soil does not affect the concentrations of PCBs in outdoor air at the height most commonly used to monitor atmospheric concentrations (typically 1-1.5 m). It also supports the findings of Jamshidi et al., who concluded using chiral signatures that the major source of PCBs to "bulk" outdoor air (defined as that sampled at 1.5 m) was ventilation of indoor air and not volatilisation from soil (Jamshidi et al., 2007).

It can be seen in figure 3.1 that there were higher concentrations of ∑PCBs in air in the 2009 campaign compared to the 2010 campaign. This may be accounted for by the difference in the time of year the samples were taken. The first samples in 2009 were taken from the 3rd June-

25th September when average air temperature recorded on the University of Birmingham campus was 15.3 ˚C compared to those recorded in the 2010 campaign from 26th March-18th June when average temperature recorded was 10.9 ˚C. This is consistent with previous observations of higher concentrations in air during warmer periods, where it has been observed in a number of studies that there are seasonal variations in the atmospheric concentrations of POPs (Halsall et al., 1995; Wania et al., 1998; Currado, and Harrad 2000; Harrad and Mao, 2004; Bozlaker et al., 2008). One possibility for this is that warmer temperatures will result in increased volatilisation of PCBs from soil. In our study, although there was a decrease in overall concentrations between the two sampling campaigns the lowest concentrations were still observed closest to the ground in both years. A possibility could be that the major source of PCBs in outdoor air is ventilated indoor air and that the summer increase in concentration is due to greater building ventilation during the warmer summer months, with the opposite effect occurring in colder months. The influence of indoor air on outdoor air concentrations has been suggested by Currado and Harrad who estimated the major source of tri- and tetrachlorinated PCBs in outdoor air to be ventilation of indoor air (Currado and Harrad 2000). Halsall et al., also suggested that PCBs in buildings was the greatest source to outdoor air (Halsall et al., 1995). PCB concentrations have been found to be significantly lower in outdoor air compared to indoor air, with concentrations reported in indoor air from Birmingham of between 540-44800 pg/m^3 (Hazrati and Harrad, 2006). Therefore indoor air can potentially be a major contributing source to outdoor air concentrations.

3.4 Enantiomer fractions of PCBs 95 and 136

The EFs of PCBs 95 and 136 were determined, with results shown in tables 3.5 and 3.6 respectively. The racemic or near-racemic EFs of PCB 95 recorded in air at all except the

lowest height samples concurred with previous findings (Robson and Harrad, 2004, Jamshidi et al., 2007). There was found to be appreciable deviation from racemic of PCB 95 in all of the soil samples of 0.452±0.006 in 2009 and 0.447±0.007 in 2010. This is similar to that seen at the same site of 0.453 ±0.023 in 2001-2002 (Robson and Harrad, 2004).

Table 3.5 Enantiomer fractions of PCB 95 in soil, grass, and air at different heights

Year	Sampling Day	SOIL	GRASS	AIR (3 cm)	AIR (10 cm)	AIR (40 cm)	AIR (90 cm)	AIR (130 cm)
2009	15	0.445	0.462	0.459	0.500	0.497	0.503	0.506
	29	0.458	0.466	nd	0.507	0.512	0.498	0.497
	44	0.456	0.467	0.449	0.501	0.496	0.501	0.502
	58	0.443	0.463	nd	0.504	0.496	0.491	0.502
	72	0.450	0.475	0.447	0.499	0.496	0.493	0.508
	85	0.453	0.474	0.451	0.506	0.494	0.499	0.500
	100	0.462	0.467	0.450	0.486	0.496	0.496	0.497
	114	0.448	0.469	0.459	0.499	0.498	0.502	0.498
	MEAN	**0.452**	**0.468**	**0.453**	**0.500**	**0.498**	**0.498**	**0.501**
	σ_{n-1}	0.006	0.004	0.005	0.006	0.006	0.004	0.004
2010	14	0.440	0.482	0.467	0.489	0.499	0.504	0.501
	28	0.439	0.479	0.468	0.488	0.498	0.501	0.501
	42	0.456	0.465	nd	nd	0.501	0.499	0.501
	56	0.445	0.487	0.470	0.490	0.500	0.500	0.499
	70	0.454	0.494	0.468	0.489	0.499	0.499	0.505
	84	0.446	0.494	nd	0.489	0.495	0.501	0.504
	MEAN	**0.447**	**0.483**	**0.468**	**0.489**	**0.499**	**0.501**	**0.502**
	σ_{n-1}	0.007	0.011	0.002	0.001	0.002	0.002	0.002

Table 3.6 Enantiomer fractions of PCB 136 in soil, grass, and air at different heights

Year	Sampling Day	SOIL	GRASS	AIR (3 cm)	AIR (10 cm)	AIR (40 cm)	AIR (90 cm)	AIR (130 cm)
2009	15	0.524	0.520	0.517	0.505	0.505	0.505	0.501
	29	0.521	0.531	0.501	0.503	0.505	0.508	0.510
	44	0.517	0.502	nd	0.502	0.503	0.503	0.501
	58	nd	0.507	nd	0.494	0.516	0.505	0.512
	72	0.510	0.515	0.517	0.510	0.505	0.506	0.501
	85	0.516	0.516	nd	0.498	0.503	0.507	0.500
	100	0.512	0.528	nd	0.507	0.499	0.501	0.507
	114	0.526	0.519	nd	0.505	0.506	0.510	0.497
	MEAN	**0.518**	**0.517**	**0.511**	**0.503**	**0.505**	**0.505**	**0.504**
	σ_{n-1}	0.006	0.010	0.009	0.005	0.005	0.003	0.005
2010	14	0.512	0.515	0.515	0.508	0.502	0.506	0.500
	28	0.505	0.517	0.518	0.508	0.503	0.497	0.508
	42	0.502	0.521	nd	nd	nd	0.504	0.502
	56	0.507	0.518	nd	nd	0.502	0.497	0.502
	70	0.516	0.493	nd	0.500	0.503	0.506	0.503
	84	0.501	0.501	nd	nd	0.508	0.502	0.495
	MEAN	**0.507**	**0.511**	**0.516**	**0.505**	**0.504**	**0.502**	**0.502**
	σ_{n-1}	0.006	0.011	0.002	0.005	0.003	0.004	0.004

nd= not detected

For PCB 136 there was observed to be a slight enrichment of the (+) enantiomer in the soil samples from 2009 which was also seen in previous studies – e.g. an EF of 0.522±0.012 has been detected at EROS (Robson and Harrad, 2004). This deviation was less evident in the 2010 soil samples. There was also observed to be a slight deviation from racemic in the grass and 3 cm air although PCB 136 was not detected in the majority of the 3 cm air samples. In

conclusion, the deviation from racemic of 136 in soils is slight and therefore cannot be used to determine the relative contribution of soil-borne PCBs to those detected in grass.

3.5 Comparison of the enantiomer fractions of PCB 95 in soil, grass and air and its implications for source apportionment.

Figures 3.2 and 3.3 show the average EFs of PCB 95 in soil, air and grass at air at various heights from the ground. In both sampling campaigns there is a clear deviation from racemic in the air samples collected closest to the ground, close to that seen in soil samples. A t-test shows that the EFs in air at 3 cm differ significantly ($p<0.05$) from those at the other heights sampled in both 2009 and 2010. The results therefore indicate that PCBs at this site volatilise from soil at a level that is discernible only in air very close to the soil:air interface. Finizio et al., conducted a similar experiment for chiral OCs α-HCH, HEPT, HEPX and o,p'-DDT in soils and air from graduating heights of 5, 35, 75 and 140 cm from the ground (Finizio et al., 1998). For HEPT, HEPX and o,p'-DDT it was observed that the chiral signatures at all heights matched closely with that seen in soil. This is different from the observations in this study where only the lowest height was affected, although it should be noted that the concentrations in the Finizio et al. study were higher (42 to 945 ng/g dw) than our study (310-1030 ∑PCBs pg/g dw). For α-HCH a trend was shown with height with the greatest deviation being closest to the ground, matching closely with that seen in soil and a gradual trend towards racemic with height which was similar to the observations in our study.

It was also observed that the EFs in grass deviate from racemic, particularly in the summer 2009 samples. This suggests that the origin of PCBs in grass could stem from dry gaseous foliar uptake of PCBs volatilised from soil. Another possibility for this deviation could be biotransformation within the grass itself. A recent study has found that poplar plants may take

up PCB 95 enantioselectively from a hydroponic solution (Zhai et al., 2011). There is substantial inter-species variation in how plants translocate PCBs from root to foliage and while translocation cannot be ruled out for the grass samples the results do not suggest this. Firstly PCB 95 was not shown to translocate to the upper stem or leaves of poplars (Zhai et al., 2011). Also the EF shift observed for PCB 95 in poplars was to a lower EF value than in the hydroponic solution. This is inconsistent with the higher EFs observed in grass than soil in this study. It is possible that grass may display opposite enantioselective preference for PCB 95 than poplars, thereby shifting the EF to a higher value than that present in soil. However, to be consistent with our observations, such enantioselective behaviour by grass would have to be more facile at lower temperatures. This is because the differential between EF values in soil and grass was greater in the colder 2010 campaign. Therefore the evidence from our data suggests PCBs in grass arise substantially from dry gaseous foliar uptake of PCBs volatilised from soil.

There is a greater difference in EFs between the soil and the 3 cm height air collected in 2010 compared to 2009. This may be attributed to the fact that the 2009 samples were collected in the summer (average temperature 15.3 ^{o}C) whereas the 2010 sampling took place in the spring (average temperature 10.9 ^{o}C) when the soil temperatures will be lower. There was also less deviation from racemic in the grass 2010 samples which may also be due to the time of year the samples were taken. This is supported by findings by Harrad et al., who found in a seasonal experiment that the EFs of PCB 95 in grass were near racemic at the start of the growing season in March and then approached those found in top soil at the same site until the end of growing season before returning to near racemic at the end of winter (Harrad et al., 2006). It was shown by Ayris et al., in artificially contaminated soils that temperature has an important influence on the edaphic persistence of PCBs (Ayris et al., 1999). Figure 3.4 shows

how the EFs varied over the 16 weeks sampling in 2009 and 12 week sampling period in 2010. It shows the grass EFs are found to be closer to that seen in soil and air sampled at 3 cm than that in "bulk" air sampled at 130 cm.

Figure 3.2: Chiral Signatures (Average ± σ_{n-1}) of PCB 95 in Samples of Air, Soil, and Grass taken in 2009

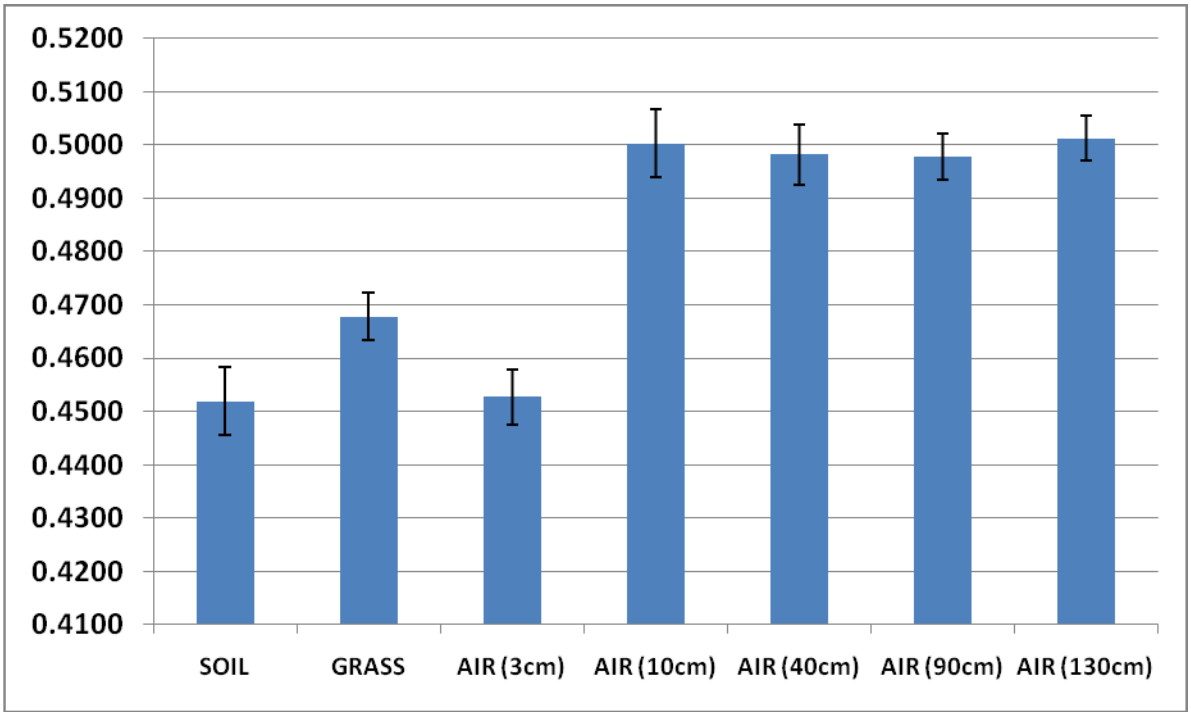


Figure 3.3: Chiral Signatures (Average ± σ_{n-1}) of PCB 95 in Samples of Air, Soil, and Grass taken in 2010

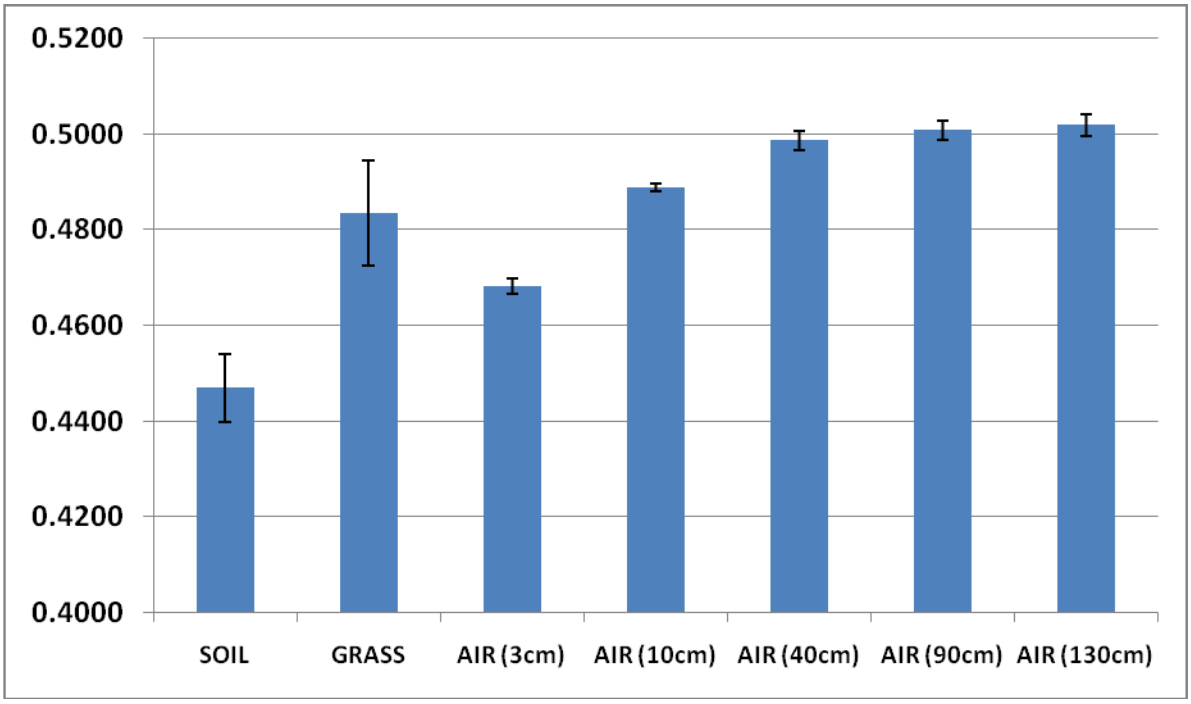


Figure 3.4 : EFs of PCB 95 in Samples of Air at 3 cm and 130 cm height, Soil, and Grass taken in 2009

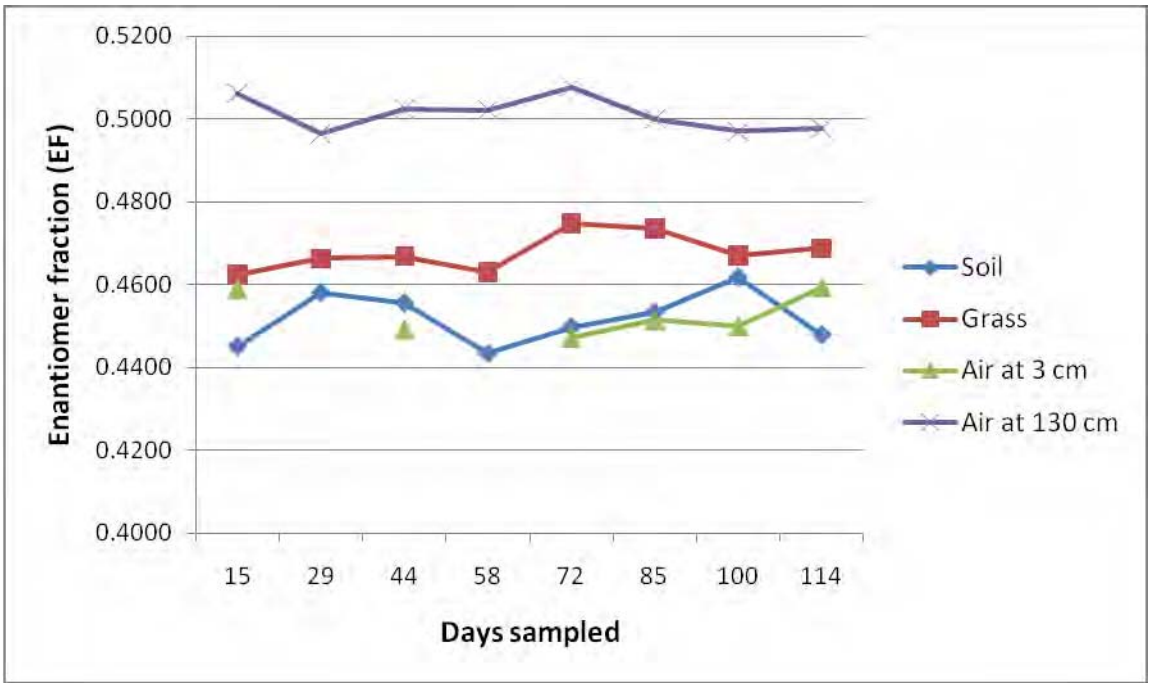
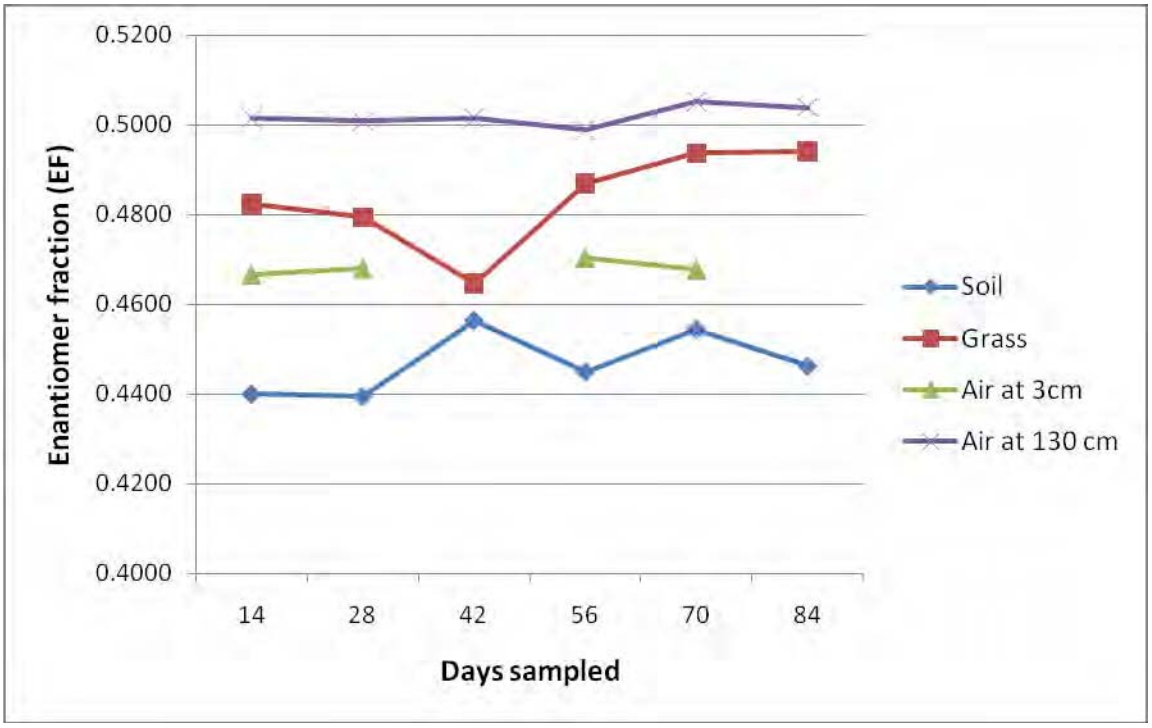


Figure 3.5 : EFs of PCB 95 in Samples of Air at 3 cm and 130 cm height, Soil, and Grass taken in 2010



3.6 Active sampling

Active air sampling was also undertaken over 50 days at 3 cm height from the ground to ascertain the EFs of PCBs 95 and 136 as well as the concentration in air at this height which could not be confirmed using PAS. The results are shown in table 3.7 and show there is also deviation from racemic of PCB 95 as seen in the 3 cm PAS samples. This supports the data from the PAS and indicates that volatilisation from soil affects the concentrations in air close to the air:soil interface.

Table 3.7 Enantiomer fractions and deviation from racemic of active air sampled 3 cm from the ground.

		∑PCBs (pg/m^3)	EFs		Deviation from racemic	
			95	136	95	136
2009	gas phase	45	0.470	0.515	0.031	-0.015
	particle	5.7	0.462	nd	0.038	nd
2010	gas phase	36	0.470	nd	0.030	nd
	particle	1.7	nd	nd	nd	nd

The gas phase ∑PCB concentrations from active sampling are 45 and 36 pg/m^3 for 2009 and 2010 respectively which are lower than that seen in the 10 cm PAS of 88 pg/m^3 and 60 pg/m^3. This is also an indication that there is an increase in concentration of ∑PCBs in air the further away from the ground and is unlikely to be a difference in sampling rate due to position of the samplers in relation to the ground.

3.7 Two source apportionment model for PCB 95

A two source apportionment model can be used to quantify the relative contributions of two different sources to a sample and can be calculated according to equation 3.1 (Harner et al., 2000).

$$f_1 = (EF_{MIX} - EF_2) / (EF_1 - EF_2) \qquad \text{(Equation 3.1)}$$

Where f_1 is the fractional contribution of source 1, EF_{MIX} is the enantiomer fraction in the "receiving" matrix and EF_1 and EF_2 are the two contributing sources.

Using this equation we can estimate the relative contribution of soil (this study) and indoor air (Jamshidi et al., 2007) on the receiving matrix of PCBs in outdoor at 3 cm (this study). It was calculated that the contribution of soil to air at 3 cm was 98% in 2009 and 57% in 2010.

Secondly we can estimate the relative contribution of air at 3 cm height and "bulk" air at 130 cm height on PCBs in grass. Air at 3 cm height was estimated to account for 69% of PCBs in grass in 2009 and 53% in 2010. These two estimates can then be combined to give an estimate for the contribution of soil-borne PCBs to grass, which gives an estimate of 68 % in 2009 and 30% in 2010.

3.8 Percentage contributions of the PCB congeners in air, grass and soil.

Care was taken to prevent any inadvertent contamination of the lowest height PUF disk and grass samples with soil particles. To check there was not any contamination we compared the congener profiles in soil with those detected in grass and air samples. Table 3.8 shows that while profiles in air and grass were dominated by PCBs 28/31 and 52 with an average of 69% of ΣPCB in air and an average of 41% ΣPCB in grass, these congeners constituted only an average of 9% of ΣPCB in soil. This implies that any contamination of both the PUF disks and grass with soil was minimal, and that the chiral signatures detected in the lowest height air samples and in grass reflect accurately what was present in these samples.

Table 3.8 Percentage contributions to ΣPCB of PCB congeners in air sampled at 3 cm, soil and grass samples

		% Percentage contribution						
		PCB 28+31	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180
AIR (3 cm)	Mean	40	29	13	5	5	5	2
	σ_{n-1}	12	9	5	2	3	3	2
	Min	25	11	9	3	2	2	0
	Max	59	43	29	10	11	10	7
SOIL	Mean	5	4	11	14	26	33	8
	σ_{n-1}	2	2	2	2	2	2	4
	Min	2	2	9	12	22	29	2
	Max	11	9	17	17	28	36	16
GRASS	Mean	21	20	14	9	17	13	6
	σ_{n-1}	7	7	4	2	4	4	2
	Min	14	12	9	6	11	8	3
	Max	39	30	20	13	22	19	9

3.9 McLachlan modelling framework for predicting primary uptake processes

A plot of log (C_V/C_G) vs Log K_{OA} can be used for identifying the primary process of plant uptake for more volatile POPs. The McLachlan framework is detailed in section 1.2.3.1 and the plots are illustrated in figure 1.3. The McLachlan framework is a method of determining the uptake mechanism of POPs into vegetation. Log (C_V/C_G) can be plotted against Log K_{OA} where C_V is the concentration of PCBs in grass and C_G is the concentration in air. If the uptake of PCBs by grass is driven by equilibrium then the slope of the plot will be linear. Using K_{OA} values for the PCB congeners from Li et al., 2003. Two graphs for each year were plotted, one using the air concentrations at 3 cm obtained from active sampling and one using the concentrations of PCBs at 130 cm from the ground. The plots for 2009 are shown in figures 3.6 and 3.7 and the 2010 plots are shown in figures 3.8 and 3.9.

The graphs show statistically significant linear relationships in 2009 using the data from air at 3 cm ($R^2 \geq 0.87$) and at 130 cm ($R^2 \geq 0.81$) and in 2010 for air at 3 cm ($R^2 \geq 0.84$). This indicates the uptake of PCBs by grass is driven by equilibrium partitioning. In 2010 there was not seen to be a significant linear relationship between 70 days and 84 days using the air data at 130 cm with R^2 of 0.39 and 0.50 repectively.

The slopes for the 2009 graphs are greater than those in the corresponding 3 cm air and 130 cm air graphs in the 2010 campaign. The average slope being 0.80 and 0.58 for 2009 and 2010 respectively using 3 cm data and 0.43 and 0.26 for 2009 and 2010 respectively using 130 cm data. This is likely to be due to the difference in temperature between the two sampling campaigns. Lower temperatures increase K_{OA} and drives the grass:air equilibrium towards grass. This might also explain the higher grass concentrations in the 2010 campaign, although the soil concentration was also higher during this campaign.

Figure 3.6: Plot of Log(C_V/C_G) vs Log K_{OA} using 3 cm air data in 2009

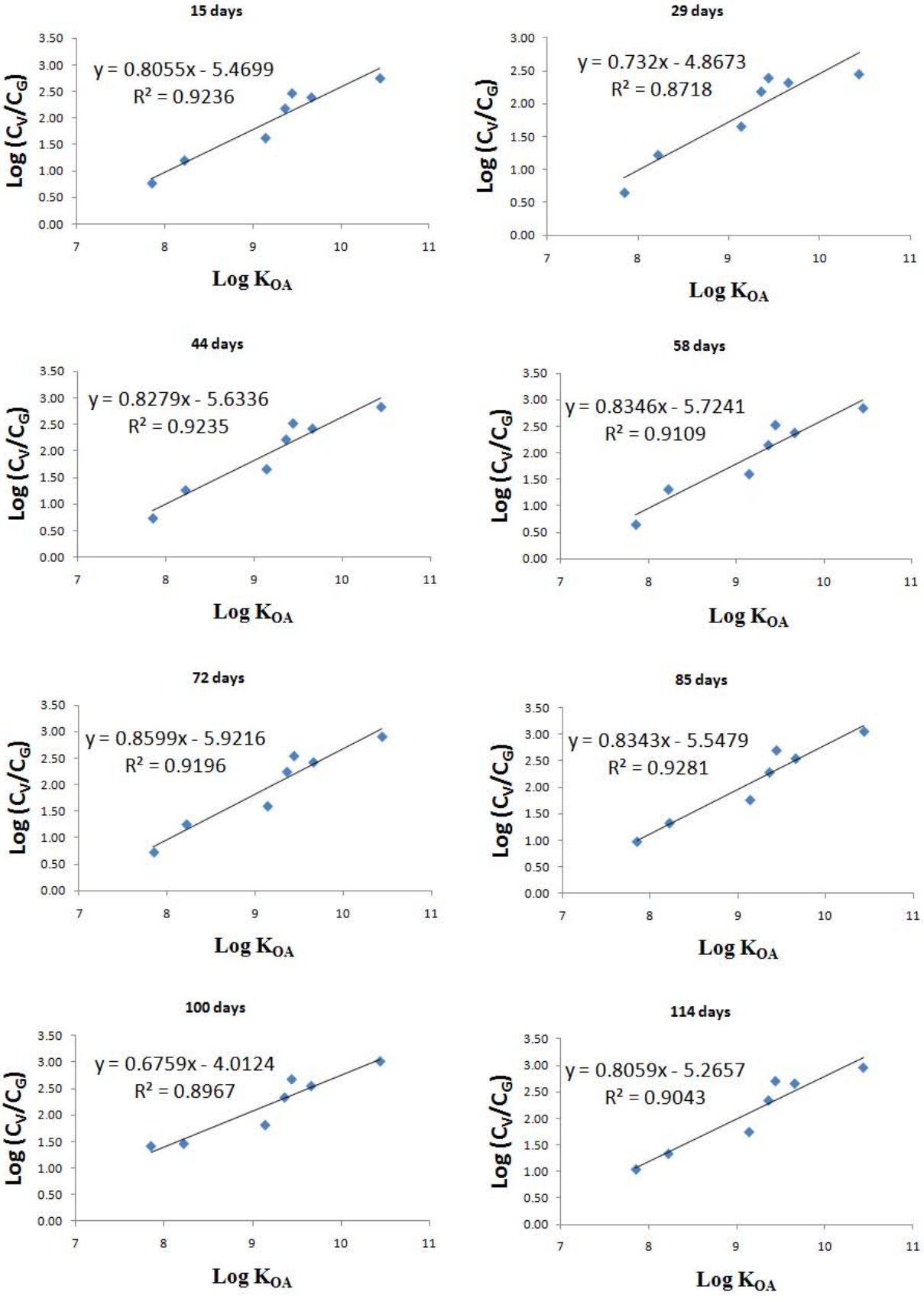


Figure 3.7: Plot of Log(C_V/C_G) vs Log K_{OA} using 130 cm air data in 2009

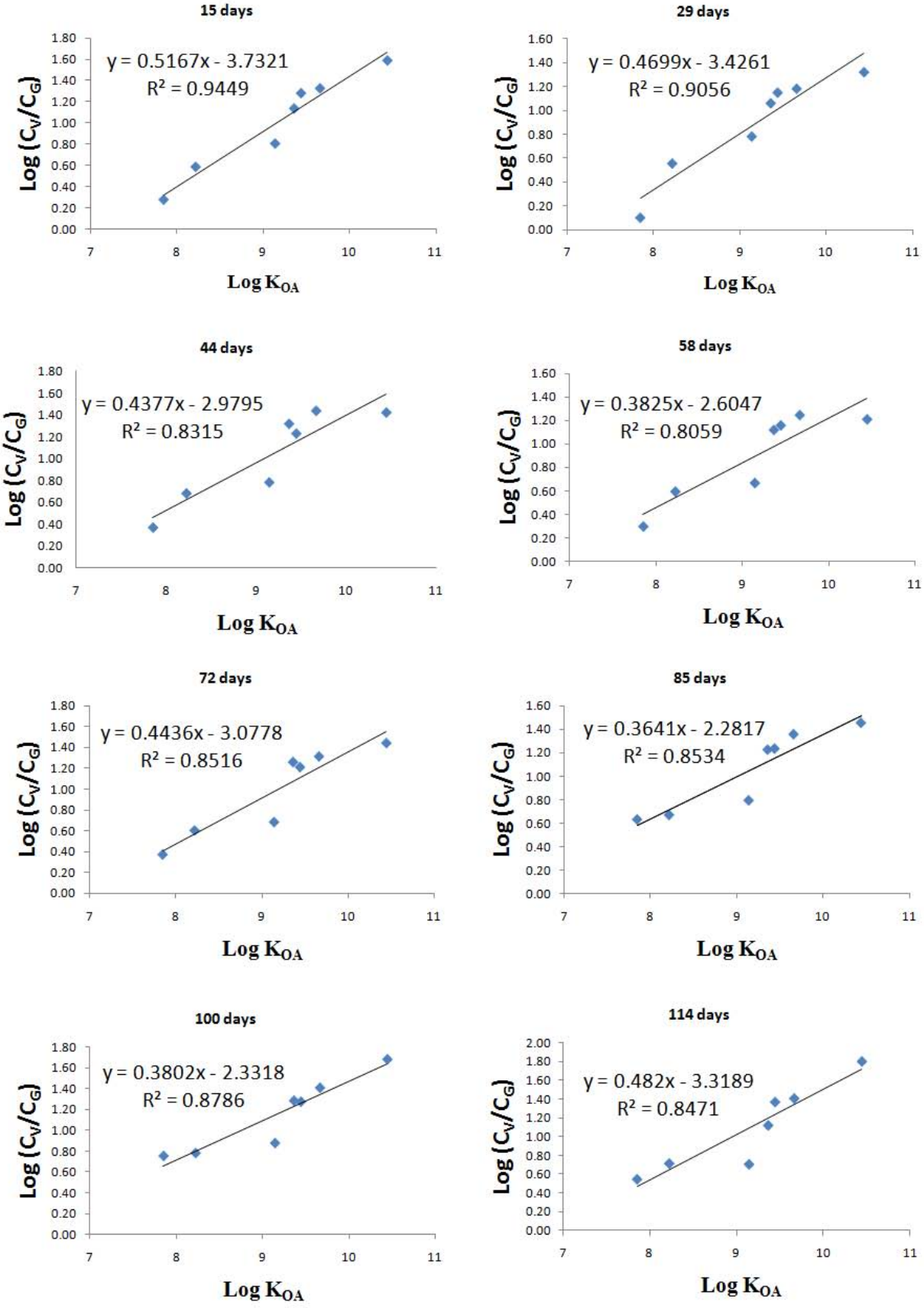


Figure 3.8: Plot of Log(C_V/C_G) vs Log K_{OA} using 3 cm air data in 2010

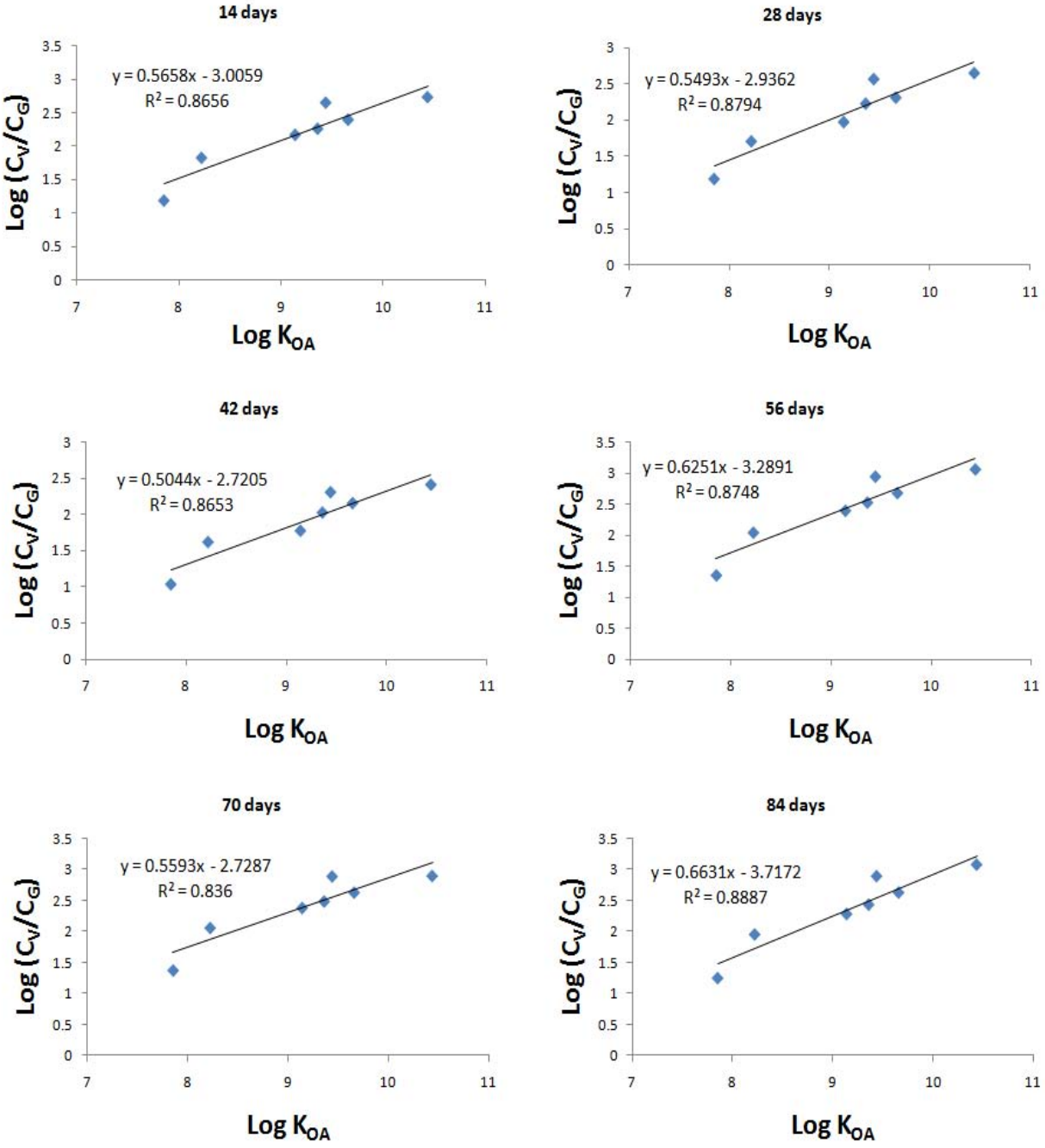
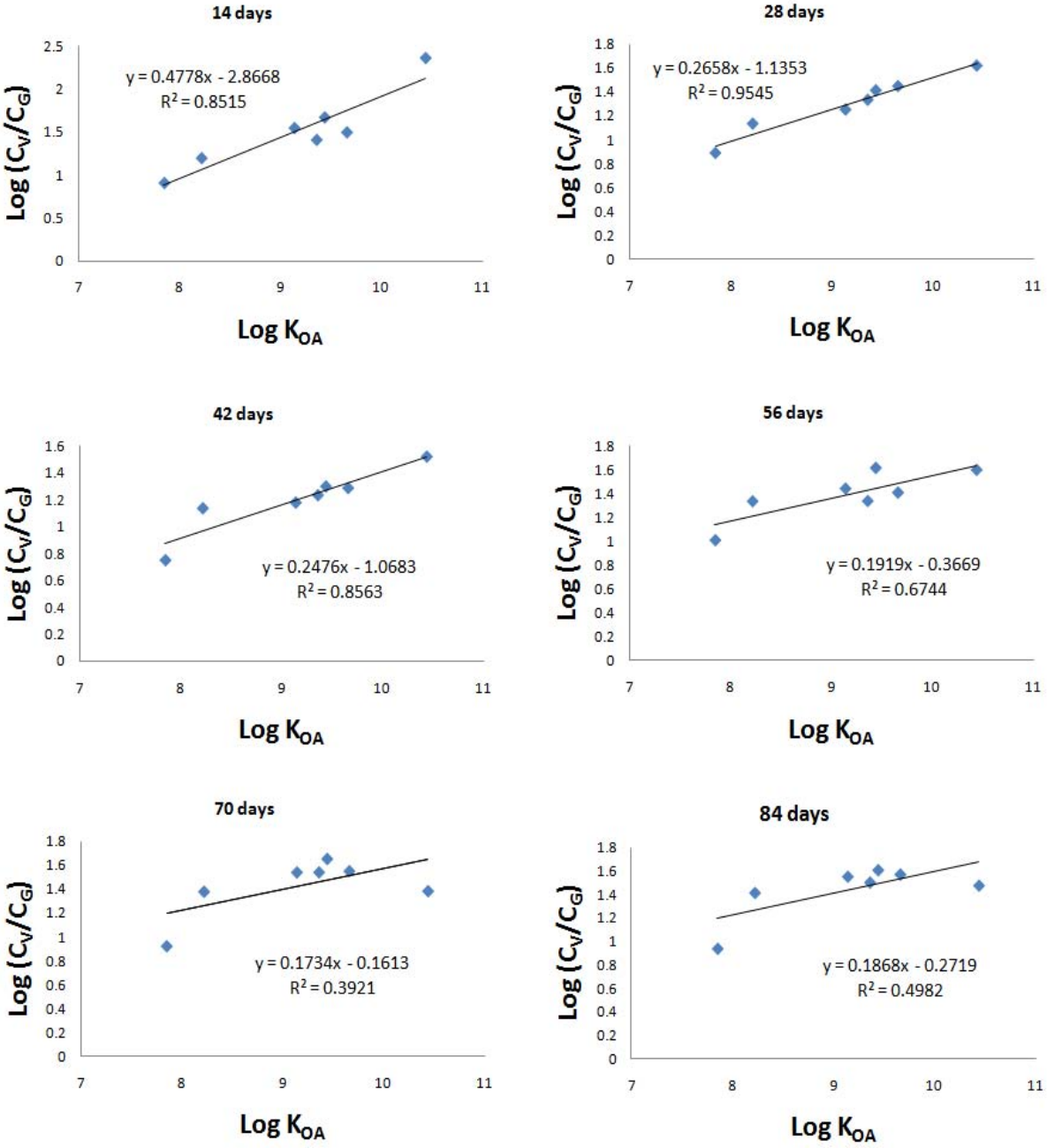


Figure 3. 9 Plot of Log(C_V/C_G) vs Log K_{OA} using 130 cm air data in 2010



3.10 Summary

Concentrations and enantiomer fractions of PCBs were determined in soil, air at different heights and grass samples. Chiral signatures of PCBs were compared in these samples to see if they could be used to provide insights into the sources of PCBs in grass.

There was found to be an increase in atmospheric concentrations of PCBs with height which suggests that at this site volatilisation from soil does not exert an appreciable influence on the concentration of PCBs in "bulk" air – defined here as air sampled at 10 cm and above. The chiral signature data showed there to be a deviation from racemic of PCB 95 in soil samples as well as in air measured at 3 cm from the surface. In contrast, chiral signatures in air sampled at 10 cm and above were racemic or near-racemic. This suggests that PCBs do volatilise from soil but at the soil concentrations in this study, this is discernible only very close to the soil surface. This combined with the observed increase in concentration with height suggests that indoor sources are the main source to outdoor air. This also supports the findings from previous studies (Jamshidi et al., 2007).

There was also observed to be a deviation from racemic in some grass samples which was particularly marked in samples from 2009 which were taken in the summer at the height of the growing season. This implies that PCBs in grass may be due to vapour phase foliar uptake of PCBs from soil. Another possibility could be biotransformation within the grass. PCB 95 has been shown to be enantioselectively taken up by poplar plants, although it was not shown to translocate to the upper stem or leaves (Zhai et al., 2011). The EF shift observed for PCB 95 in poplars was to a lower EF which is inconsistent with the higher EFs observed in grass from our study suggesting that enantioselective uptake in grass is unlikely. Using a two source apportionment model it was estimated that air at 3 cm height accounted for 69% of PCBs in grass in 2009 and 53% in 2010. The contribution of soil-borne PCBs to grass was estimated to be 68% in 2009 and 30% in 2010. This difference could be due to the time of year that the samples were taken and the resulting differences in temperature.

The suggestion from these results is that volatilisation from soil is potentially an important route via which PCBs and potentially other POPs with similar vapour pressures can enter the food chain. The increase in concentration with heights combined with chiral signature data for air at 3 cm to the ground indicates that the urban site monitored in this study, indoor sources are the main source to "bulk" outdoor air but not to the air that supplies grass at the soil:air interface. Combined with the chiral signature data, this suggests the influence of PCB emissions from soil on airborne concentrations decreases with altitude, while that of emissions from the built environment increases. This raises the interesting point that while removing PCBs from buildings will reduce our inhalation exposure, it may take longer to see a reduction in dietary exposure. There are important implications of these findings for the biogeochemical cycling of PCBs. It highlights an important mechanism via which the substantial reservoir of PCBs in soils might be transferred into the terrestrial food chain.

CHAPTER 4: CONCENTRATIONS AND CHIRAL SIGNATURES OF HBCDs IN SOILS, GRASS, AND AIR FROM THE UK AND IN SOILS FROM AUSTRALIA.

4.1 Synopsis

This chapter looks at the concentrations of HBCDs and their chiral signatures in the environment. HBCDs were determined in soil, grass and outdoor air at EROS in Birmingham UK. HBCDs and their chiral signatures were also determined in soils from 24 sites from around the UK and from 17 sites in Australia. Additional soil samples were also taken from the UK site with the highest concentration of HBCD to examine the influence of a suspected source of HBCD contamination. There are currently very few data available on the levels of HBCD in soils. This is a potentially significant omission, given the likely role of soil as a source of HBCD to the terrestrial food chain. The aims were:

1. to determine if HBCDs display evidence of enantioselective degradation in soils, and if so to see how edaphic chiral signatures compare with those in air and grass;
2. to determine the concentrations of HBCDs in soil from different sites around the UK to test the hypothesis that they will be ubiquitous in UK soils consistent with the situation for other POPs such as PCBs;
3. to examine diastereoisomer profiles in these soils to determine if there was deviation from the profile of the commercial HBCD formulation and to test the hypothesis that outdoor samples may deviate further from the commercial mixture than observed previously for indoor samples due to the differences in temperature and potential for photolysis;
4. to construct a preliminary environmental budget for HBCDs in the UK environment;

5. to determine concentrations of HBCDs in soils from around Australia;

6. to determine the diastereoisomer profiles in these soils to test the hypothesis that these samples may deviate further from the commercial mixture than UK soil samples due to the different climatic conditions; and

7. to determine the enantiomer fractions in these soils to see if there was any evidence of enantioselective degradation of HBCDs in Australian soils.

4.2 Sampling strategy

4.2.1 Air sampling

Air was analysed using PUF disk passive air samplers (PAS), for which the sampling rates were 1.66, 1.58 and 1.70 m^3 day^{-1} for α, β, and γ-HBCD respectively; determined by a 50 day calibration experiment as detailed in section 2.5.7.

4.2.2 Soil and grass sampling

Soil was sampled as detailed in section 2.2.3. To summarise briefly, the soil was taken from the top 5 cm from four different points in a 1 m x 1 m area and homogenised before analysis. Twenty of the UK samples were taken between 10th December 2004-4th March 2005 and 7th June 2005-19th September 2005 (Evans, 2007). Additional soil samples were taken from EROS, Birmingham and London in 2008 and 2009. The Australian soils were collected as part of the national dioxin program in 2002/3. They were sampled from the top 10 cm from 4 sub-sampling sites that were combined to form one sample per site. The UK grass samples were taken from a 1 m^2 area at EROS in 2010 and freeze dried after collection as specified in

section 2.2.4. The soils and grass were extracted and prepared for analysis according to sections 2.3.1.2 and 2.3.1.3 respectively. All samples were analysed for HBCDs using LC-MS/MS as outlined in section 2.4.1.

4.3 Concentrations and enantiomer fractions of HBCD from Birmingham UK

4.3.1 Concentrations and enantiomer fractions of HBCDs in air, grass and soil

Concentrations were measured in air using PUF disc samplers at EROS on a vertical transect at 5 heights from the ground over 28 days. The heights were 10 cm, 54 cm, 100 cm, 122 cm, and 142 cm for 28 days, with samples collect every 14 days. Concentrations of HBCDs were also determined in air sampled using a PUF disc sampler deployed at 1.5 m height every 14 days over a 3 month period in 2010.

The concentrations of HBCDs and their enantiomer fractions were determined in air, grass, and soil at EROS and are shown in tables 4.1, 4.2, and 4.3. Similar concentrations were seen in the soil and grass with mean concentrations of 0.39±0.12 and 0.27±0.11 ng/g ∑HBCD for soil and grass respectively.

Table 4.1 Concentrations of HBCDs in soil from EROS (ng/g)

	Concentration ng/g				%∑HBCD			Enantiomer fraction		
Date collected	α-HBCD	β-HBCD	γ-HBCD	∑HBCD	α-HBCD	β-HBCD	γ-HBCD	α-HBCD	β-HBCD	γ-HBCD
03/06/2008	0.23	0.04	0.17	0.44	52	9	39	0.502	0.514	0.534
	0.29	0.05	0.17	0.51	57	10	33	0.511	0.520	0.538
20/10/2008	0.10	0.03	0.22	0.35	29	9	63	0.489	0.483	0.507
	0.10	0.02	0.12	0.24	42	8	50	0.498	0.467	0.466
Mean	0.18	0.035	0.17	0.39	45	9	46	0.500	0.496	0.511
σ_{n-1}	0.10	0.013	0.04	0.12	13	1	13	0.009	0.025	0.033

Table 4.2 Concentrations of HBCDs in Grass from EROS (ng/g)

	Concentration ng/g				%∑HBCD			Enantiomer fraction		
Date collected	α-HBCD	β-HBCD	γ-HBCD	∑HBCD	α-HBCD	β-HBCD	γ-HBCD	α-HBCD	β-HBCD	γ-HBCD
09/04/10	0.13	0.05	0.23	0.42	32	13	55	0.502	<dl	0.505
23/04/10	0.08	0.04	0.15	0.28	30	15	55	0.502	<dl	0.503
07/05/10	0.05	0.03	0.09	0.17	32	16	52	<dl	<dl	<dl
21/05/10	0.09	0.03	0.11	0.23	39	12	49	<dl	<dl	<dl
Mean	0.09	0.04	0.15	0.27	33	14	53	0.502	<dl	0.504
σ_{n-1}	0.033	0.012	0.063	0.11	4	2	3	0	<dl	0.001

Table 4. 3 Concentrations of HBCDs in air sampled on a vertical transect at EROS

	Concentration pg/m^3				%			Enantiomer fraction		
Sampler Height (cm)	α-HBCD	β-HBCD	γ-HBCD	∑HBCD	α-HBCD	β-HBCD	γ-HBCD	α-HBCD	β-HBCD	γ-HBCD
03/07/08-17/07/08										
10	44	20	62	125	35	16	49	0.525	0.461	0.507
54	32	19	41	92	35	20	45	0.489	0.468	0.503
100	44	22	22	88	50	25	25	0.465	0.505	0.507
122	43	23	24	89	48	26	27	0.479	0.477	0.467
142	34	17	21	72	47	24	29	0.483	0.484	0.472
17/07/08-01/08/08										
10	20	8.7	18	46	43	19	38	0.490	0.528	0.487
54	14	9.0	16	38	36	23	40	0.484	0.498	0.481
100	18	<dl	16	34	54	1	46	0.469	<dl	0.563
122	19	<dl	35	54	36	1	64	0.513	<dl	0.492
142	21	15	27	63	33	24	43	0.508	0.478	0.493

The air samples contained between 38 and 125 pg/m^3 ∑HBCD. This is only slightly higher than the average ∑HBCD concentration of 37±2 pg/m^3 reported from the same site (Abdallah et al., 2008c). Table 4.5 shows outdoor air from this study compared to others around the world. The percentage contribution of the diastereoisomers in the air varied between the samples with γ-HBCD accounting for between 25-64%. Grass contained the greatest amount of γ-HBCD and there was little variation over the 8 week growing period with the average percentage contribution of the three diastereoisomers being 33±4%, 14±2% and 53±3% of α-, β- and γ-HBCD respectively. Li et al determined HBCDs in cabbage and radishes grown in spiked soils observed there to be predominantly α-HBCD, compared to soil they were grown in which contained predominantly γ-HBCD suggesting there was either diasteroisomer specific translocation or selective metabolism within the plant (Li et al., 2011). This difference is likely to be due to interspecies variation and the small number of samples in our study.

None of the HBCD enantiomers in samples showed appreciable deviation from racemic in any of the samples of air, soil, or grass in this study. It was therefore evident that enantioselective degradation had not occurred and a study design as detailed in chapter 3 for PCBs could not be conducted for HBCDs at this site. There are limited data on enantioselective degradation of HBCD in the environmental samples. It was found in air sampled from a city in China that there was enrichment of (-)-α-HBCD suggesting possible soil-air exchange of HBCDs (Yu et al., 2008b). Enantioselective degradation of HBCD in soils is discussed further in section 4.4.3.

4.3.2 Concentrations of HCBD in air from EROS 2010

There are limited data on the concentrations of HBCD in outdoor air in the UK and indeed elsewhere. Therefore HBCD was measured in air at EROS in Birmingham using PAS at 1.5 m height over a 4 month period starting on 26th March 2010 with samples being collected every 14 days. The results are shown in table 4.4.

Table 4.4 HBCD contamination of outdoor air sampled at 1.5 m height in spring/summer 2010

Date	Concentration pg/m^3				%∑HBCD		
	Alpha	Beta	Gamma	∑HBCD	Alpha	Beta	Gamma
26/03-09/04	12	5.5	18	35	34	16	50
09/04-23/04	8.6	3.9	12	24	36	16	48
23/04-07/05	11	4.3	27	42	25	10	64
07/05-21/05	5.5	2.2	7.0	15	37	15	48
21/05-04/06	5.3	3.1	7.7	16	33	19	48
04/06-18/06	7.5	2.6	31	41	18	6	75
18/06-02/07	3.4	2.4	9.5	15	22	15	62
02/07-16/07	5.4	3.6	12	21	25	17	58
Mean	**7.3**	**3.5**	**15**	**26**	**29**	**14**	**57**
σ_{n-1}	**2.9**	**1.1**	**9.0**	**12**	**7**	**4**	**10**
Median	**7**	**3**	**12**	**23**	**29**	**16**	**54**
Min	**3**	**2**	**7**	**15**	**18**	**6**	**48**
Max	**12**	**6**	**31**	**42**	**37**	**19**	**75**

Figure 4.1 HBCD concentrations over 4 months sampling in 2010

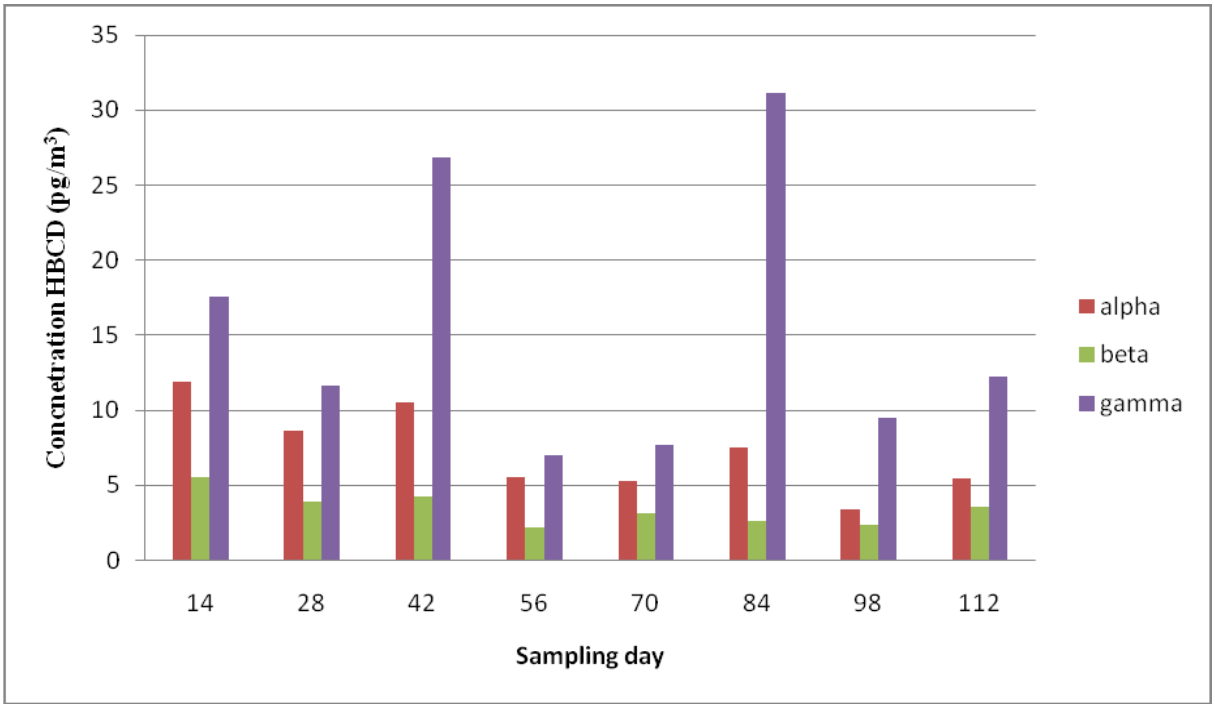


Figure 4.1 shows the concentrations measured over the 2010 sampling campaign. The average concentration of ∑HBCD over the four month campaign was 26±12 pg/m^3 ranging between 15-42 pg/m^3. This is in good agreement with active air sampling at the same site in 2007 (n=5) which reported an average ∑HBCD concentration of 37±2 pg/m^3 (Abdallah et al., 2008c). Compared to indoor air the levels in this study are considerably lower. Abdallah et al., reported an average level of ∑HBCD of 250 pg/m^3 from 33 homes, 180 pg/m^3 from 25 offices and 900 pg/m^3 in public microenvironments (PMEs) (Abdallah et al., 2008c). This is likely to be due to the enclosed environment and a greater number of HBCD emission sources being present in indoor environments such as building insulation, textiles and electronics.

Data for HBCD in outdoor air from the literature are shown in table 4.5. The concentrations in Birmingham are slightly higher than that seen in US outdoor air where concentrations were reported as <0.1-11 pg/m^3 ∑HBCD (Hoh and Hites, 2005), in two Swedish sites where levels were found to be 5.3 and 6.1 pg/m^3 ∑HBCD (De Wit, 2002) and in Japan where

concentrations from two sites outside homes were 13 and 15 pg/m^3 ∑HBCD (Takigami et al., 2007). In contrast, the levels in this study are below those measured in urban air from Sweden and within the range seen in remote locations in Sweden and Finland of 2-280 pg/m^3 ∑HBCD (Remberger et al., 2004).

Table 4.5 Comparison of HBCD in outdoor air from around the world

Location	Details	∑HBCD pg/m^3 Mean (range)	Reference
Birmingham UK	Urban (n=8)	26 (15-42)	This study
Birmingham UK	Urban (n=5)	37 (34-40)	Abdallah et al., 2008c
USA	Particulate matter	(<0.1-11)	Hoh and Hites, 2005
Japan	Residential (n=2)	(13-15)	Takigami et al., 2007
Sweden	(n=2)	(5.3-6.1)	de Wit, 2002
Sweden/Finland	exhaust of XPS ventilation system (n=1)	1070000	Remberger et al., 2004
	Landfill (n=2)	13-180	
	Textile industry (n=2)	19-740	
	Urban (n=2)	76-610	
	Remote (n=6)	2-280	

The predominant diastereoisomer in all outdoor air samples was γ-HBCD which constituted between 48-75% of ∑HBCD (average 57%). This was followed by α-HBCD which comprised 18-37% ΣHBCD (average 29%), with the remainder being β-HBCD which accounted for between 6-19% ΣHBCD (average 14%). This represents a slight shift from γ-HBCD to α-HBCD compared to that found in the commercial mixture. There is a shift from γ-

HBCD to α-HBCD when HBCDs are incorporated into products at temperatures exceeding 160 ^{o}C where the diastereoisomers are liable to rearrangement (Köppen et al., 2008). The deviation could therefore be from HBCDs leaching directly from products treated with HBCD. It could also be indicative of the difference in vapour pressure as α-HBCD has been measured to have a vapour pressure an order of magnitude higher than γ-HBCD (Kuramochi and Sakai, 2010). Indoor air from Birmingham UK was found to contain 22% of α-HBCD and 65% of γ-HBCD (Abdallah et al., 2008c). This was a slightly higher proportion of γ-HBCD than seen outdoors in this study which could indicate photo-mediated isomerisation is occurring in the outdoor environment. However, a much more detailed study involving considerably larger sample numbers is required to investigate this hypothesis fully.

4.4 Concentrations of HBCD diastereoisomers and enantiomers in UK soils

4.4.1 Concentrations of HBCDs in UK soils

HBCDs were determined in 23 soil samples from the UK. Table 4.6 shows HBCD contamination in UK soils studied displayed substantial spatial variability with concentrations of ∑HBCD ranging from 0.07 to 424 ng/g. Average concentrations were 3.9, 1.8, 18, and 23 ng/g for α-, β-, γ-, and ΣHBCDs respectively. Notwithstanding the particularly high concentration found at the London site, the levels found are comparable to those seen in soils from the West Midlands of both ∑tri-hexa-BDEs (0.07-3.9 ng g^{-1}) and ∑PCBs (0.57-13.3 ng g^{-1}) (Harrad and Hunter 2006, Jamshidi et al., 2007). Like these compounds HBCDs are now ubiquitous in the environment as a result of their widespread use. The highest concentration (424 ng/g ∑HBCDs) was found at a suburban location in London, with the lowest concentration (0.07 ng/g ∑HBCDs) detected in Scoat Tarn, a remote rural location. Table 4.7 shows the average concentrations of the sites divided into urban, surburban and rural sites.

The suburban and urban samples contain on average higher concentrations than the rural samples. This suggests the existence of an urban/suburban increment in HBCD concentrations as seen previously for PBDEs and PCBs (Harrad and Hunter 2006, Jamshidi et al., 2007).

Table 4.6 HBCD in soils from the UK

			Concentration ng/g				%∑HBCD			Enantiomer fraction		
Location	Land use	Year collected	α-HBCD	β-HBCD	γ-HBCD	∑HBCD	α-HBCD	β-HBCD	γ-HBCD	α-HBCD	β-HBCD	γ-HBCD
Birmingham (n=2)[a] summer	Urban	2008	0.26	0.05	0.17	0.48	54	10	35	0.506	0.517	0.536
Birmingham (n=2)[a] Winter	Urban	2008	0.10	0.03	0.22	0.35	29	9	63	0.494	0.475	0.486
Edinburgh	Urban	2005	9.3	1.4	4.5	15	61	9	30	0.501	0.464	0.504
Worcester	Urban	2005	1.9	3.2	39	44	4	7	88	0.503	0.518	0.466
Aberdeen	Urban	2005	0.26	0.06	0.45	0.77	34	8	58	0.498	0.495	0.528
Preston	Urban	2005	0.10	0.12	0.23	0.45	22	27	51	<dl	<dl	<dl
Southampton	Urban	2005	0.58	0.22	3.9	4.7	12	5	83	0.531	<dl	0.494
UEA Campus	Urban	2005	0.23	0.09	0.88	1.2	19	8	73	<dl	<dl	0.506
Rugby	Urban	2005	0.07	<0.01	0.17	0.24	29	2	69	<dl	<dl	0.495
Saffron Walden	Urban	2005	0.19	0.11	0.69	0.99	19	11	70	0.510	<dl	0.498
Brewood	Suburban	2005	0.42	0.23	0.94	1.6	26	14	59	0.468	0.487	0.479
Daventry	Suburban	2005	0.19	<0.01	0.09	0.28	67	2	32	0.515	0.483	0.508
Keele	Suburban	2005	0.25	0.08	0.84	1.2	21	7	72	0.484	<dl	0.511
Lancaster	Suburban	2005	5.1	1.2	15	21	24	6	70	0.512	<dl	0.513
Essex	Suburban	2005	0.25	0.03	0.37	0.65	38	5	57	0.531	0.513	0.482
Wales (Llandudno)	Suburban	2005	0.09	0.05	0.16	0.30	30	17	53	<dl	<dl	0.492
Cornwall (Helston)	Suburban	2005	0.12	0.05	0.52	0.69	17	7	75	0.518	0.503	0.502
London	Suburban	2009	59	28	340	420	14	7	79	0.498	0.474	0.509
Stevenage	Suburban	2010	0.79	0.24	1.6	2.6	30	9	61	nm	nm	nm

Bushmills	Rural	2005	<0.01	<0.01	<0.01	<0.03	<dl	<dl	<dl	<dl	<dl	<dl
Norfolk	Rural	2005	5.7	0.77	1.4	7.9	72	10	18	0.490	0.491	0.488
York	Rural	2005	0.12	0.03	0.11	0.26	46	12	42	0.543	0.559	0.518
Suffolk	Rural	2005	0.06	0.02	0.14	0.22	27	9	64	<dl	0.544	0.510
Scoat Tarn	Rural	2005	<0.01	<0.01	0.07	0.07	6	6	88	<dl	<dl	0.515
			Concentration ng/g				%∑HBCD			Enantiomer fraction		
			α-HBCD	β-HBCD	γ-HBCD	∑HBCD	α-HBCD	β-HBCD	γ-HBCD	α-HBCD	β-HBCD	γ-HBCD
Mean			**3.5**	**1.5**	**17**	**22**	**31**	**9**	**60**	**0.506**	**0.502**	**0.502**
σ_{n-1}			**12**	**5.7**	**69**	**86**	**18**	**5.2**	**19**	**0.019**	**0.028**	**0.017**
RSD %			**340**	**380**	**400**	**390**	**60**	**58**	**31**	**3.7**	**5.6**	**3.3**
Min			**<0.01**	**<0.01**	**<0.01**	**<0.03**	**4**	**2**	**18**	**0.468**	**0.464**	**0.466**
Max			**59**	**28**	**340**	**420**	**72**	**27**	**88**	**0.543**	**0.559**	**0.536**
5th percentile			**0.01**	**0.01**	**0.07**	**0.09**	**6.9**	**2.3**	**30**	**0.480**	**0.470**	**0.479**
95th percentile			**8.8**	**2.9**	**35**	**41**	**66**	**16**	**87**	**0.534**	**0.550**	**0.528**

[a]where more than one sample was analysed, the values given are the average.
For the purposes of statistics the non-detected concentration used was 0.5 x LOD, where LOD =blank concentration
nm- not measured
<dl = below detection limit

Table 4.7 Average concentrations (ng/g) of ∑HBCD in the urban, suburban and rural locations

	Mean	σ_{n-1}	5th percentile	95th percentile	min	Max	Median
Urban (n=10)	6.8	14	0.29	31	0.25	44	0.88
Suburban (n=9)	50	140	0.29	263	0.29	424	1.2
Rural (n=5)	1.7	3.5	0.026	6.3	<0.03	7.9	0.22

For the purposes of statistics the non-detected concentration was 0.5 x LOD

Compared to values reported in other studies of HBCDs in soil, the average concentration of ∑HBCD in the UK soils were considerably higher than that seen in soil from Chinese surface soils of 23 pg/g ∑HBCD (n=22) (Meng et al., 2011). They were however lower than samples taken close to point sources in Swedish soil of 567 ng/g ∑HBCD (Remberger et al., 2004) and soils close to HBCD manufacturing plants of 4292 ng/g ∑HBCD (n=5) (Petersen et al., 2004). Concentrations in soils were higher on average than UK sediments for which concentrations of 0.88-4.8 ng/g ∑HBCD have been reported (Harrad et al., 2009b). However, they are fairly consistent with these levels when the particularly high concentration seen in the suburban London sample is excluded. This is also true of sediments from the Detroit River, USA which contained concentrations between <0.075 and 3.7 ng/g ∑HBCD (Marvin et al., 2006) and Dublin Bay, Ireland with concentrations between <1.7 and 12 ng/g ∑HBCD (Morris et al., 2004).

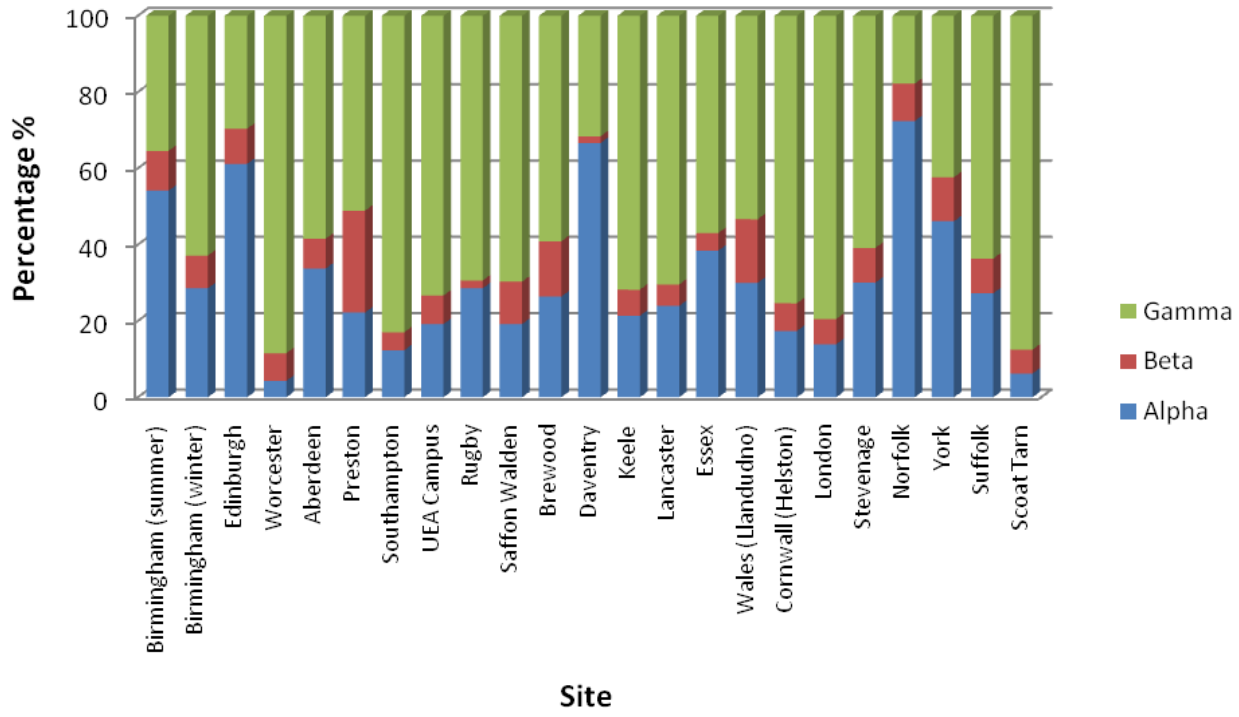
4.4.2 Diastereoisomer profiles of HBCD in UK soil

The average percentage contribution of each diastereoisomer to ΣHBCDs in the soil samples studied was 31% α-HBCD (range 4-72%), 9% β-HBCD (range 2-27%) and 60% γ-HBCD (range 18-88%). The proportions of β-HBCD were fairly consistent with that found in the commercial mixture with it being the least abundant isomer in all but the Preston sample which contained 27% β-HBCD compared to 22% α-HBCD and 51% γ-HBCD. While the majority of the samples were predominantly γ-HBCD consistent with the pattern observed in the commercial mixture; 5 of the samples contained predominantly α-HBCD, suggesting some isomerisation of γ- to α-HBCD in these soils). Interestingly, the highly contaminated London sample had a diastereoisomer profile very close to the commercial mixture. This could imply that the contamination is recent.

These variations in diastereoisomer profiles have also been observed in a number of other studies into soils and sediments. Yu et al. found in soils from China that two samples showed patterns closer to the commercial product and one showed a dominance of the alpha isomer (Yu et al., 2008a). Gao et al., analysed soils from e-waste and industrial sites in China and reported α-HBCD as the most predominant isomer in 37 of the 90 samples (Gao et al., 2011). A study on nine UK lakes found sediments to contain on average 75% γ-HBCD, (range 61-80%) (Harrad et al., 2009b). Such diastereoisomer patterns are closer to the commercial mixture compared to the UK soils. Although the UK soils are from 24 sites compared to 9 sites for the sediment samples which could account for the greater variation in soils; this could be due to more facile photolytic or aerobic bioisomerisation in surface soils compared to sediments. It was observed in sediments from the Detroit River that γ-HBCD was the predominant isomer in approximately two thirds of the samples, whereas α-HBCD was predominant in the other third of samples (Marvin et al., 2006). Morris et al., found the

majority of sediments from North Sea estuaries to be predominantly γ-HBCD (Morris et al., 2004). They did however observe higher percentages of both the α- and β-HBCD in some Belgian locations and an absence of α and γ-HBCD was observed in sediment from the River Mersey in the U.K.

Figure 4.2: The percent contribution of HBCD diastereoisomers to ∑HBCDs in UK soils



4.4.3 Enantiomer fractions of HBCD diastereoisomers in UK soil

The EFs of each of the three HBCD diastereoisomers were found to be close to racemic in all samples with the average±σ_{n-1} EFs being α-HBCD 0.506±0.019, β-HBCD 0.502±0.028, and γ-HBCD 0.502±0.017. This implies there is no edaphic enantioselective degradation in these soils. There are currently limited data on EFs of HBCDs in soils and sediments. A recent study by Gao et al., found there to be no deviation from racemic in 48 soils from e-waste recycling and industrial sites (Gao et al., 2011). In the case of sediments Harrad et al., found

racemic signatures in samples from nine lakes in the UK (Harrad et al. 2009b). In the same study it was found that the majority of fish samples from the same lakes showed an appreciable enrichment of (-)α-HBCD, suggesting that *in vivo* metabolism could be responsible for this change. However Guerra et al., saw a slight enrichment of (+)-α-HBCD and (+)-γ-HBCD in sediment samples from Spain where there was a known point source of HBCDs (Guerra et al., 2008). Wu et al., also found non-racemic signatures for β- and γ-HBCD (but not α-HBCD) in suspended particles and sediment from the vicinity of an e-waste recycling plant in south west China (Wu et al., 2010).

4.4.4 Levels of HBCD in soil from a suburban site

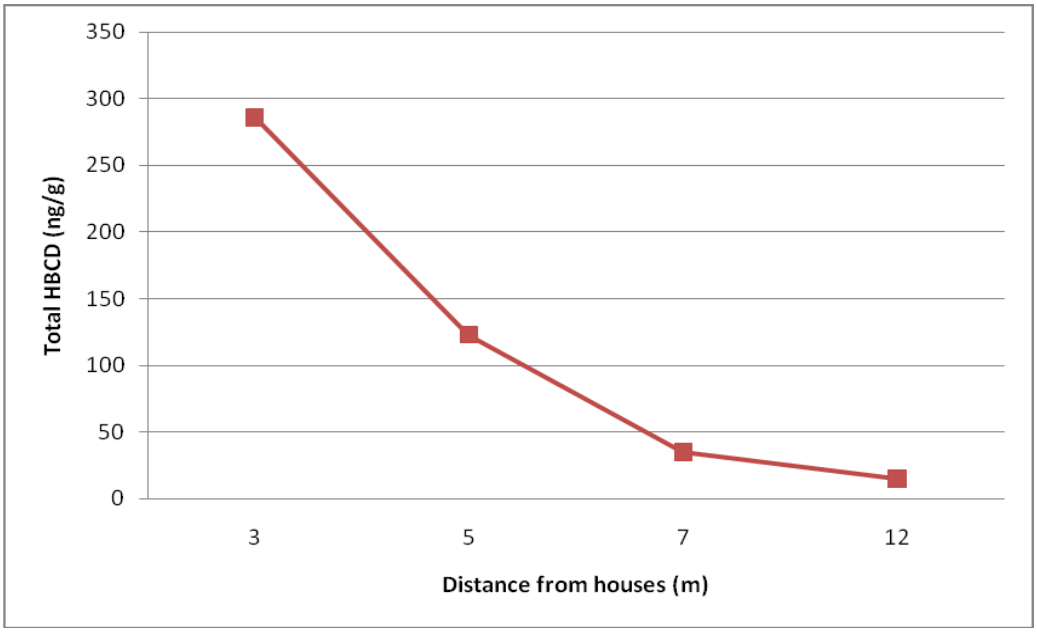
Further samples were taken from the London site due to the high levels of HBCDs (420 ng ΣHBCD/g) that were found there. The site was a suburban garden. The samples were taken on a transect across the garden with increasing distance from housing. From the results shown in table 4.8 it is clear that HBCD concentrations decrease substantially with increasing distance from the house. This implies that the source of contamination was either the house (a known use of HBCD is to flame retard expanded polystyrene thermal insulation) or from discarded building waste (e.g. thermal insulation material) buried close to the house. The levels of the three diastereoisomers found in the samples are all consistent with that found in the commercial mixture with gamma accounting for between 78-87% of the HBCDs. This close resemblance to the commercial mixture may suggest that the contamination is recent.

Table 4.8 Concentrations of HBCDs in soil samples from a London garden (ng/g)

Distance from Housing (m)	Concentration (ng/g)						Percentage contribution to ∑HBCDs		
	α-HBCD	β-HBCD	γ-HBCD	∑HBCD	PBCD	TBCD	α-HBCD	β-HBCD	γ -HBCD
3	35	28	223	286	3.0	0.90	12	10	78
5	15	10	98	123	1.1	0.09	12	8	80
7	4.5	2.7	28	35	0.17	0.30	13	8	80
12	1.3	0.6	13	15	<0.01	<0.01	9	4	87

For house dust Harrad et al., observed that a source of contamination came from a television set (Harrad et al., 2009a). Samples taken closest to the television set contained the highest levels of HBCD which dropped greatly the further away the sample was taken, there was also a significant shift from predominantly γ-HBCD closest to the set to α-HBCD as the distance from the TV increased. Unlike in the dust samples, the percentage of α-HBCD, β-HBCD and γ-HBCD in our outdoor soil samples were found to be consistent despite the concentrations dropping by more than half between each of the samples. This is likely to be due to the difference in the sample matrix and the fact that the dust study was indoors rather than outdoors. It is possible that HBCDs in soil below the surface will be more shielded from solar radiation, so less susceptible to photolysis than those bound to dust. Also the source of the contamination is unknown in the soil samples but is suspected to have arisen from the house due to the decrease in contamination with increasing distance. Outdoor temperatures will also be lower reducing the potential for thermal degradation.

Figure 4.3: Graph showing total HBCD (ng/g) in samples taken in 2010 at increasing distances from housing



4.5 Estimated burdens of HBCDs in the UK environment

Using data from this study along with data from other studies from different environmental compartments it is possible to estimate the burden of HBCD to the UK environment. This study used the same approach used by Harrad et al., to estimate the burden of PCBs to the UK environment (Harrad et al., 1994).

Air

For this study as for the Harrad et al. study the surface area of the UK was taken to be 2.475 x 10^{11} m^2 (Geo-data 1983 cited in Harrad et al., 1994). It was assumed that the atmospheric mixing height was 1 km above the surface. Concentrations above are assumed insignificant to POPs burden. As rural HBCD air data is not currently available it was estimated using the ratio of the average urban: rural concentrations found in UK soils and then applied to the

urban air data. Using estimates of UK land use (Geo data cited in Harrad et al., 1994) we assumed that 90% of the UK is contaminated at this level with the remaining contamination estimated to be at the level measured in Birmingham UK.

Table 4.9 HBCD concentrations and estimated burden in UK air

Diastereoisomer	Mean concentrations in UK air pg/m^3		UK atmospheric burden (kg)
	Rural[a]	Urban	
α-HBCD	0.61	7.3	0.32
β-HBCD	0.29	3.5	0.15
γ-HBCD	1.3	15	0.67
∑HBCD	2.2	26	1.1

[a]estimated values, see main text for explanation

Soils

The average HBCD concentrations for UK soil from this study used to estimate the burden. Using the estimated surface area of the UK to be 2.475 x 10^{11} m^2 (Geo-data 1983 cited in Harrad et al., 1994) and average soil bulk density as 1000 kg m^{-3}, the top 5 cm of UK topsoil will weigh 1.24 x 10^{13} kg it is possible to estimate the burden of HBCD to soil. The estimates are shown in table 4.10 and show there to be a considerable burden of HBCD associated with soils. This may be an underestimation as we are only taking into account the top 5 cm of the soil surface, although we are assuming the greatest concentrations will reside in this layer.

Table 4.10 HBCD concentrations and estimated burden in UK soil

Diastereoisomer	Mean concentrations in UK soils (μg/kg)	UK soil burden (kg)
α-HBCD	3.5	43,000
β-HBCD	1.5	19,000
γ-HBCD	17	210,000
∑HBCD	22	270,000

Grass

The burden of HBCD associated with grass in the UK was estimated using data from EROS from this study. HBCD in grass was estimated by assuming a dry aerial biomass of 260 g m^{-2} (Haygarth et al., 1991). It will therefore be estimated that the vegetation in the UK weighs 6.4 x 10^{10} kg. There are substantial limitations to this estimation as it is based on concentrations from just one urban site and a small number of samples. The estimates are shown in figure 4.11.

Table 4.11 HBCD concentrations and estimated burden in UK grass

Diastereoisomer	Mean concentrations in UK grass (μg/kg)	UK grass burden (kg)
α-HBCD	0.09	5.8
β-HBCD	0.04	2.6
γ-HBCD	0.15	9.6
∑HBCD	0.27	17

UK population

Data from human milk samples was used to estimate the human burden (Abdallah and Harrad 2011). The UK population was taken to be approximately 59 million according to the 2001

census (ONS accessed 2011). It was estimated based on an average adult weighing 70 kg and having 25% body fat (US, EPA, 1997).

Table 4.12 HBCD concentrations and estimated burden in UK population

Diastereoisomer	Mean concentrations in UK population (μg/kg)	UK human burden (kg)
α-HBCD	4.91	5.1
β-HBCD	0.32	0.33
γ-HBCD	0.73	0.75
∑HBCD	5.95	6.1

Lake water

Data for UK lake water was used to estimate the HBCD burden associated with UK freshwater bodies (Harrad et al., 2009b). The burden of HBCDs in UK lakes was determined by assuming the mean depth of lakes to be 10 m and that freshwater bodies constitute 1% surface area of the UK which is 2.5 x 10^9 m^2, and results in an estimated total freshwater lake volume of 2.5 x 10^{10} m^3 (Geo data 1983 cited in Harrad et al., 1994).

Table 4.13 HBCD concentrations and estimated burden in UK lake water

Diastereoisomer	Mean concentrations in UK lakes[a] (pg/L)	UK lakes burden (kg)
α-HBCD	37	0.93
β-HBCD	19	0.48
γ-HBCD	100	2.5
∑HBCD	160	4.0

[a] sum of both dissolved and particulate phase (Harrad et al., 2009b)

Lake sediments

The surface area occupied by freshwater bodies in the UK was assumed to be 2.5 x 10^9 m^2 (see above). The data we used to estimate the sediment burden was from UK lakes (Harrad et al., 2009). The depth of the surficial sediments associated with these freshwater bodies was assumed to be 26 cm as used by Harrad et al., for the PCB estimation as we are assuming HBCD will have accumulated in sediments below the 5 cm surface (Harrad et al., 1994). Assuming a sediment density of 130 kg m^{-3} the weight of sediment was taken to be 8.45 x 10^{10} kg.

Table 4.14 HBCD concentrations and estimated burden in UK lake sediments

Diastereoisomer	Mean concentrations in UK lake sediments (μg/kg)	UK lake sediment burden (kg)
α-HBCD	0.30	26
β-HBCD	0.24	21
γ-HBCD	1.7	140
∑HBCD	2.2	190

Total estimated burdens of HBCDs in the UK environment

The estimated burdens associated with each of the environmental compartments are shown in table 4.15. The greatest sink is soils, followed by sediments. This is consistent with the lipophilic properties of HBCD which allows it to preferentially bind to particulate matter. The same was seen for PCBs where soil was also estimated to be the greatest sink in the UK environment (Harrad et al., 1994). It should be noted that are uncertainty in these estimations. Firstly the grass and air samples are only based on data on one site. The soil estimation also only takes into account the top 5 cm of soil where it is assumed the greatest burden of HBCD

will reside, although it is possible that this is an underestimation. Although there are limitations to these estimations it gives a preliminary indication of the main sinks for HBCDs in the terrestrial UK environment. A significant gap in this estimation is the lack of data for HBCD in sea water and marine sediments. The latter in particular are likely to constitute an important sink of HBCD in the environment and could mean a significant underestimation of the total HBCD burden.

Table 4.15 Estimated distribution of HBCD in the UK environment between different environmental compartments.

Compartment	UK burden (kg)			
	α-HBCD	β-HBCD	γ-HBCD	∑HBCD
Outdoor air	0.32	0.15	0.67	1.1
Soil	43,000	19,000	210,000	270,000
Freshwater sediments	26	21	140	190
Grass	5.8	2.6	9.6	17
Fresh water	0.93	0.48	2.5	4.0
Humans	5.1	0.33	0.75	6.1
Total burden	43038	19025	210154	270218

The total burden of ∑HBCD in the environment was calculated to be 270,000 kg which is only slightly less than that estimated in 1994 for ∑PCBs where the total burden was calculated to be 400,000 kg and from the same compartments as this study to be 375,000 kg (Harrad et al., 1994). The PCB burden today is likely to be lower than this estimation due to PCBs no longer being produced. A clear implication of this preliminary environmental budget is that migration of HBCD from products to the environment has been substantial and comparable to that observed for PCBs.

4.6 HBCDs in Australian soils

4.6.1 Concentrations of HBCDs in Australian soils

Soils from 17 locations across Australia were analysed for HBCD and the results shown in table 4.16. ∑HBCD was found in samples at concentrations ranging from 0.0048 to 5.6 ng/g with an average concentration of 0.74 ng/g. HBCDs were found predominantly in the urban and industrial soil samples. HBCD was not detected in any of the agricultural samples and found at a low level in only one of the remote samples. While HBCDs have previously been detected in the atmosphere in remote regions and in animals from polar regions (Covaci et al., 2006); their absence from agricultural and remote soils may suggest that their capacity for such long range transport is limited.

The soils were from urban, industrial, agricultural and remote locations. Table 4.16 shows the average concentrations from the different location categories. The mean concentration in the 8 urban samples was 1500 pg/g for the Australian soils which was lower than that seen in the UK urban soils of 6800 pg/g. Table 4.17 shows the concentrations from this study with others from around the world. The average concentrations in the Australian soils were lower than those seen in the UK surface soils. As there is only a small number of samples in this study there may not be a great significance to this. The levels are considerably lower than those found in studies which were taken close to point sources such as HCBD manufacturing plants (Remberger et al., 2004; Petersen et al., 2004). The mean concentration is consistent with those measured in surface soils in China (Yu et al., 2008a) and sediments from UK lakes which are not impacted by a HBCD point source which contained ∑HBCD between 880-4800 pg/g (Harrad et al., 2009b).

Table 4.16 Concentrations and Chiral Signatures of HBCDs and degradation products in Australian soil samples.

Location	Land use	Date collected	State	Concentration pg/g				Percentage %∑HBCD			Enantiomer fraction		
				α-HBCD	β-HBCD	γ-HBCD	∑HBCD	α-HBCD	β-HBCD	γ-HBCD	α-HBCD	β-HBCD	γ-HBCD
Currumbin Gold Coast	Urban	07/10/2002	QLD	49	20	320	390	12	5	82	0.497	0.504	0.494
Canberra	Urban	25/11/2002	ACT	13	1.9	24	38	33	5	62	0.498	0.486	0.496
Sydney	Urban	16/04/2003	NSW	120	64	1600	1800	7	4	89	0.499	<dl	0.497
Newcastle	Urban	07/05/2003	NSW	390	160	2400	2900	13	6	81	0.512	0.475	0.511
Woolongong	Urban	17/02/2003	NSW	120	42	1100	1300	9	3	88	0.481	<dl	0.506
Launceston	Urban	01/04/2003	TAS	470	160	4900	5600	8	3	89	0.521	0.487	0.485
Perth Duncraig	Urban	20/03/2003	WA	20	8.8	120	150	13	6	81	0.509	0.475	0.488
Perth Kings Park	Urban	06/08/2003	WA	5.8	1.9	51	58	10	3	87	0.487	<dl	0.487
Whyalla	Industrial	03/01/2003	SA	16	<0.1	79	95	17	0	83	<dl	<dl	0.532
Port Pirie	Industrial	03/01/2003	SA	21	9.4	130	160	13	6	81	0.501	0.494	0.492
Port Phillip	Industrial	01/04/2003	VIC	13	6.0	88	110	13	6	82	0.494	0.485	0.505
Kwinana	Industrial	20/03/2003	WA	6.4	1.7	31	39	17	4	79	0.509	0.498	0.505
Gympie Forestry	Agricultural	10/03/2003	QLD	<0.2	<0.1	<0.2	<0.5	<dl	<dl	<dl	<dl	<dl	<dl
Lismore	Agricultural	05/01/2002	NSW	<0.2	<0.1	<0.2	<0.5	<dl	<dl	<dl	<dl	<dl	<dl

Wagga Wagga	Agricultural	04/12/2002	NSW	<0.2	<0.1	<0.2	<0.5	<dl	<dl	<dl	<dl	<dl	<dl
Alice Springs	Remote	01/03/2003	NT	0.83	<0.1	3.9	4.8	17	1	81	<dl	<dl	<dl
Central Tasmania	Remote	01/03/2003	TAS	<0.2	<0.1	<0.2	<0.5	<dl	<dl	<dl	<dl	<dl	<dl
				Concentration pg/g				Percentage %∑HBCD			Enantiomer fraction		
				α-HBCD	β-HBCD	γ-HBCD	∑HBCD	α-HBCD	β-HBCD	γ-HBCD	α-HBCD	β-HBCD	γ – HBCD
Mean				73	28	640	740	14	4	82	0.501	0.488	0.500
Stdev				140	53	1300	1500	6	2	7	0.011	0.010	0.013
RSD %				190	190	200	200	46	46	8.3	2.3	2.1	2.6
Min				<0.2	<0.1	<0.2	<0.5	7	0	62	<dl	<dl	<dl
Max				470	160	4900	5600	33	6	89	0.521	0.504	0.532
5th percentile				0.1	0.1	0.1	0.3	7.9	0.7	72	0.484	0.475	0.486
95th percentile				410	160	2900	3400	24	5.8	89	0.516	0.502	0.520

For the purposes of statistics the non-detected concentrations were assumed to be 0.5 x LOD

Table 4.17 Comparison of Concentrations of HBCDs in Australian and UK soils with data for soils and sediments from around the world.

Sampling site	Soil/sediment type	Mean concentration (range) ng/g dry wt.				Reference
		∑HBCD	Alpha	Beta	Gamma	
UK soils (n=24) (This study)	Urban/suburban/rural Surface soil (5cm depth)	22 (<0.03-420)	3.5 (<0.01-59)	1.5 (<0.01-28)	17 (<0.01-340)	This study
Australian soils (n=17) (This study)	Urban/industrial/remote Surface soil (10cm depth)	0.74 (<0.0005-5.6)	0.073 (<0.0002-0.47)	0.028 (<0.0001-0.16)	0.64 (<0.0002-4.9)	This study
Sweden soil (n=3)	Close to XPS producing plant	567 (140-1300)	-	-	-	Remberger et al., 2004
Belgium/ Germany soil (n=5)	Close to HBCD manufacturing plants	4292 (111-23200)				Petersen et al., 2004
China (n=90)	E-waste and industrial areas	(0.01-284)	(0.00-61.5)	(0.00-12.7)	(0.01-215)	Gao et al., 2011
UK Lake sediment (n=9)	Sediment (5 cm depth)	(0.88-4.8)	(0.11-0.62)	(0.064-0.50)	(0.66-3.8)	Harrad et al., 2009b
Sediment: Scheldt basin, Belgium (n=20)		60 (<0.2-950)	-	-	-	Morris et al., 2004
Western Scheldt (n=19)		10 (<0.6-99)	-	-	-	
Netherlands (n=9)	Estuarine/Riverine	3.2 (<0.8-9.9)	-	-	-	
Sediment: England (n=22)	Estuarine/Riverine	199 (<2.4-1680)	-	-	-	
Dublin bay Ireland (n=8)		3.3 (<1.7-12)	-	-	-	
Detroit river sediment		(<0.075-3.7)	(<0.025-1.9)	(<0.025-0.28)	(<0.025-2.3)	Marvin et al. 2006
Chongming island, China, soil (n=22)	Surface soil	0.023±0.019	0.0055±0.0047	0.0012±0.0013	0.017±0.014	Meng et al., 2011
Guangzhou, China, Urban, soil (n=3)	Urban	(1.7-5.6)				Yu et al., 2008a
China (n=7)	Close to HBCD manufacturing plant	(2.8-144.5)	14.6±3.4	11.7±1.7	73.7±4.7	Jin et al., 2009

Table 4.18 Average concentrations of ∑HBCD (pg/g) in the urban, industrial, agricultural and remote locations

	Average ∑HBCD	σ_{n-1}	5^{th} percentile	95^{th} percentile	min	max	median
Urban n=8	1500	1900	45	4600	38	5600	840
Industrial n=4	100	51	47	150	39	160	100
Agricultural n=3	<0.5	-	-	-	-	-	-
Remote n=2	2.5	3.2	0.49	4.5	<0.5	4.8	2.5

4.6.2 Diastereoisomer profiles of HBCD in Australian soils

The Australian soils were found to contain predominantly γ-HBCD followed by α-HBCD and then β-HBCD with 14%±6, 4%±2 and 82%±7 for α-, β- and γ-HBCD respectively. This is the same pattern seen in the commercial formulation. Therefore the hypothesis that the climatic conditions prevailing in Australia would result in a higher relative abundance of α-HBCD is not supported by our observations for these samples.

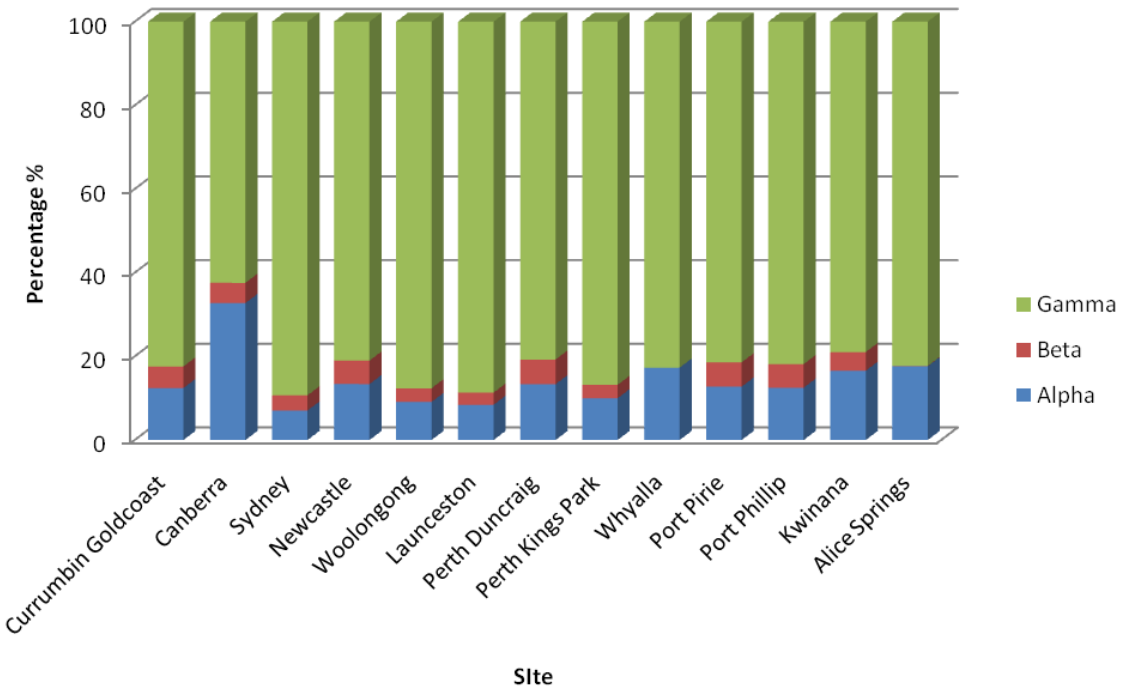


Figure 4.4 The percentage contribution of HBCD diastereoisomers to ∑HBCDs in Australian soils

Comparison with UK soils shows that there is greater variation in the UK samples with respect to diastereoisomer pattern. Figure 4.5 shows the average relative abundance of the diastereoisomers in the commercial mixture (Law et al., 2005), the Australian soils and the UK soils (this study). The UK samples deviated further from the pattern found in the commercial mixture, with 5 of the 24 samples containing predominantly α-HBCD. This conflicts with the hypothesis that samples in a warmer climate would deviate further from the commercial mixture. This could be due to the small number of samples in this study and that a larger study could show a different picture. It could be also be possible that the contamination of the Australian soils was from a fresher source and so there had not been time for appreciable degradation or isomerisation. Also possibly relevant is that the Australian samples were collected between 2002 and 2003, compared to the UK soils collected in 2005. It could

be also be possible that a commercial mixture used in Australia has a higher proportion of γ-HBCD than that used in the UK. A technical mixture of predominantly γ-HBCD at levels exceeding 98% has been reported (Ryan et al., 2006). Another possibility could be that because the Australian samples were taken from the top 10 cm of the soil compared to 5 cm in the UK samples there was less potential for photolysis in the these soils compared to the UK samples.

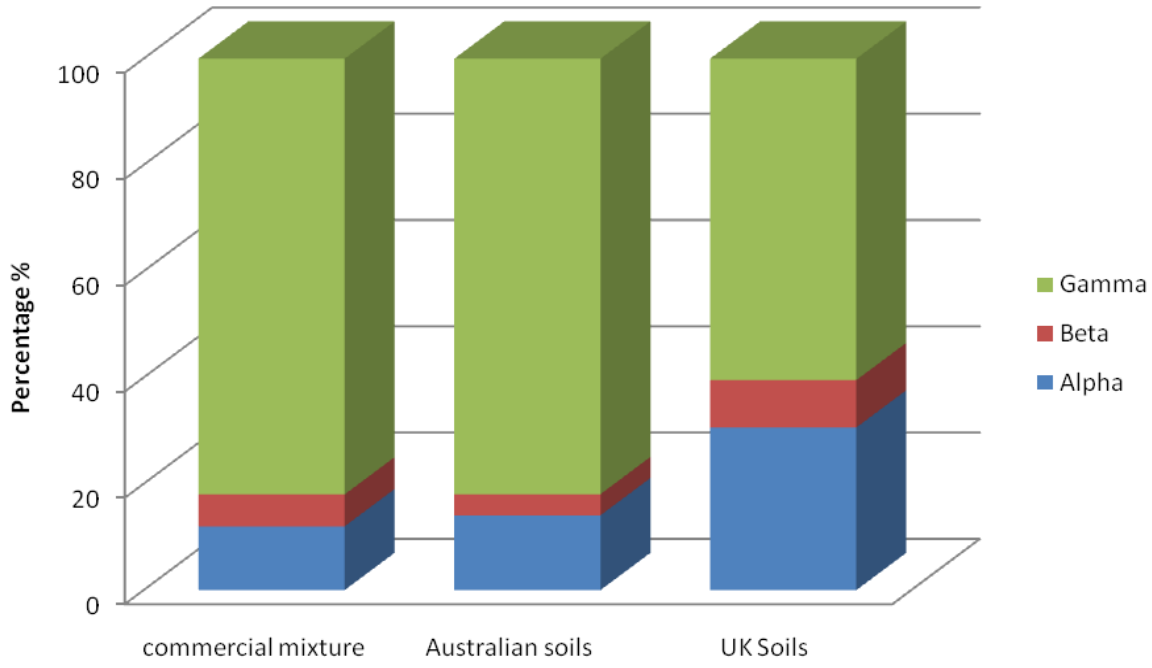


Figure 4.5 Average percentage contribution of HBCD diasteroisomers to ∑HBCD

4.6.3 Enantiomer fractions in Australian soils

As observed for UK soils, the enantiomer fractions in the Australian soils are either racemic or close to racemic in all the samples where there were measurable quantities of HBCDs. This suggests that enantioselective degradation is not enhanced in Australia despite the higher temperatures that may lead to greater microbial activity. In the case of anaerobic experiments

by Gerecke et al. on sewage sludge there was no evidence that degradation of HBCD was enantioselective (Gerecke et al., 2006). As discussed in section 4.4.3 there are limited data available on enantiomer fractions in soils and sediments. Lake sediments from the UK and soils from 48 Chinese sites were found to display racemic signatures for HBCDs, consistent with observations for UK and Australian soils in this study (Harrad et al. 2009b; Gao et al., 2011).

4.7 Summary

HBCDs and their enantiomer fractions were determined in samples of soil, grass and air at EROS in Birmingham UK. HBCDs were found to be racemic or near-racemic in all the air, grass and soil samples indicating that enantioselective degradation is not apparent at this site. Hence, while the data reported here provide valuable base-line information on the distribution of HBCDs in the environment, this site was unsuitable for a study like that used for PCBs in chapter 3 to determine the pathways of HBCD into plants using chiral signatures.

Air was also sampled over a 4 month period in spring 2010 at EROS. The concentrations in outdoor air were considerably lower than that seen in indoor air. Average outdoor air concentrations at one site in Birmingham were found to be 26±12 pg/m^3, almost an order of magnitude lower than that reported for indoor air from homes in Birmingham (Abdallah et al., 2008c). The levels are consistent with those seen in outdoor air from around the world at locations not impacted by point sources. The relative contributions of the diastereoisomers to ΣHBCDs were 29%, 14% and 57% for α-, β- and γ-HBCD respectively. This differs slightly from that observed in the commercial mixture. This might indicate that there is a thermally mediated change of the diastereoisomers when they are incorporated into products at high temperatures. Outdoor air was seen to have a slightly lower contribution of γ-HBCD to

∑HBCD concentration than seen in indoor air from Birmingham (Abdallah et al., 2008c). Although more research is required to fully evaluate this possibility, this may be indicative of a photolytic change in the outdoor environment compared to indoors.

Levels of HBCDs in soils have not been widely reported and so this is an important area of interest. These results are the most comprehensive data to date on the concentrations of HBCD diastereoisomers and their enantiomer fractions in soils. They demonstrate the ubiquity of these chemicals in the UK terrestrial environment. The levels seen are comparable to those seen in soils from the West Midlands of both ∑tri-hexa-BDEs and ∑PCBs which is of concern as it indicates substantial migration of HBCD into the environment. While the data presented here are limited in number, there is evidence of higher concentrations in urban and suburban areas than in rural and remote locations. This is consistent with the hypothesis that urban locations constitute a major diffuse source of these chemicals. The diastereoisomer patterns varied considerably between the samples. Of the 24 samples, five contained predominantly α-HBCD indicating there is isomerisation of γ-HBCD and β-HBCD to α-HBCD in some of the soil samples. This could be due to photolysis, although it is also likely that increased α-HBCD will arise as a result of thermal isomerisation during the incorporation of HBCD into products and materials.

All the enantiomer fractions were found to be close to racemic indicating there is no enantioselective degradation of HBCD in these soils. Therefore the initial intention to use HBCD chiral signatures as a means of determining pathways between air, grass and soil proved not possible for these sites. These results are similar to that seen for EFs in sediments in nine UK lakes which were also found to be racemic (Harrad et al., 2009b).

Soils from around Australia were analysed for HBCDs and their chiral signatures. The concentrations varied greatly from site to site and were found at the highest concentrations in urban areas. The concentrations were lower than those seen in the UK soil samples, although there were a relatively small number of samples and more are needed to ascertain if this difference in significant. The diastereoisomer profiles in the Australian soils are very close to that seen in the commercial mixture with γ-HBCD accounting for 62-89% of ∑HBCD in the samples. This suggests that photolytic transformation is not occurring to a great extent in the samples. A possible explanation could be that because the samples were taken from the top 10 cm of the soil there was less exposure to light and therefore less photolytic transformation between the isomers. As for UK soils, the enantiomer fractions of HBCDs in Australian samples were all found to be close to racemic implying no enantioselective microbial degradation.

A preliminary estimation of the burden of HBCD from different environmental compartments showed that the greatest burden of HBCD in the UK environment was in soils followed by sediments. This is likely due to the lipophilic nature of HBCD which allows it to preferentially bind to particulate matter such as soils and sediments. This burden is lower than that estimated for PCBs in the early 1990's of 400,000 kg (Harrad et al., 1994). Although there are limitations to these estimations it gives a good indication of the principal sinks of HBCDs in the UK environment.

CHAPTER 5: HBCD DEGRADATION PRODUCTS.

5.1 Synopsis

The degradation products pentabromocyclododecenes (PBCDs) and tetrabromocyclododecadienes (TBCDs) were determined semi quantitatively in soil samples from the UK and Australia. Degradation products were also determined in curtain samples from Japan that had been stored in the presence and absence of light. The aims of this chapter were:

1. to determine if PBCDs and TBCDs are present in soil samples from the UK to determine if the major degradation is the loss of HBr as has been observed in sediments and dust (Harrad et al., 2009b; Abdallah et al., 2008b).
2. To analyse a number of soils from Australia to test the hypothesis that the different climatic conditions will lead to different PBCD and TBCD patterns in soil and;
3. to determine the degradation products of HBCDs in textile samples which have been stored in the presence and absence of light to examine the effect of photolysis in the degradation of HBCD.

5.2 Sampling strategy

Samples were the same UK and Australian soils as those analysed for HBCD distereoisomers. They were analysed for PBCDs and TBCDs using LC-MS/MS according to section 2.4.1. As there is currently no available standards for these compounds the concentrations were semi-quantitatively calculated using the average RRFs for α-, β- and γ-HBCD. The curtain samples were provided by the National Institute for Environmental Studies, Japan.

5.3 Degradation products of HBCD in soils

5.3.1 Degradation products in soils from the UK

Two classes of degradation products (PBCDs and TBCDs) were semi quantitatively determined in 7 and 6 of the soil samples respectively with concentrations ranging from 10-7300 pg/g for ∑PBCDs and 10-1300 pg/g for ∑TBCDs. The concentrations are shown in table 5.1. There are very limited data in the literature concerning these degradation products. As far as we are aware this study is the only data currently available on PBCDs and TBCDs in top soils. A study of sediments from nine English lakes found two peaks of TBCDs at concentrations ranging from 72-810 pg/g, with PBCDs detected in 4 of the lake sediments at concentrations ranging from 37-220 pg/g (Harrad et al., 2009b). Notwithstanding the particularly high concentration of PBCD found in the London soil sample in this study the levels in soils are consistent with those seen in UK sediments.

Four PBCD peaks were identified in the soil samples. Figure 5.1 shows the chromatogram of PBCD found in the London soil sample. PBCDs has also been reported by Barontini et al. who identified seven PBCD isomers as degradation products of HBCD via GC-MS (Barontini et al., 2001a; Barontini et al., 2001b). The greater number of isomers compared to the four observed in this study may be indicative of the high temperatures employed in GC/MS compared to LC-MS/MS analysis.

The presence of PBCDs and TBCDs in soils may suggest that the loss of HBr is the major degradation pathway of HBCD. This is also the pathway suggested by Hiebl and Vetter in the case of chicken eggs and white fish where one isomer of PBCD was detected via GC-MS

(Hiebl and Vetter, 2007). Barontini et al., investigated the decomposition products of HBCD using a batch reactor and identified HBr as a gaseous deposition product with no evidence of Br_2 production (Barontini et al., 2001a). The suggestion that loss of HBr is the major mechanism of HBCD degradation conflicts with the findings of Davis et al., who found the predominant pathway of HBCD degradation to be the loss of Br_2 to give tetrabromocyclododecene (TBCDe) as the principal degradation product.

Another possibility is that these products are already present in HBCD commercial formulations as impurities. Tetrabromocyclododecene has been found to be an impurity in commercial HBCD which is present in significant quantities (Barontini et al., 2001a). A technical product of HBCD was analysed for PBCD and TBCD. It was found to contain both the PCBDs (four peaks) and TBCDs (2 peaks) shown in figures 5.6 and 5.7 respectively. This implies that these compounds are present in products before they are released into the environment.

Figures 5.1 and 5.2 show PBCDs in a London soil sample from this study and an indoor dust sample (Harrad et al., 2009). They both show a similar pattern of four peaks of PBCD, with the soil sample containing a higher proportion of the second PBCD peak compared to the dust. The presence of four peaks is different from that seen by Abdallah and Harrad in samples of human milk shown in figure 5.3 where only 3 PBCD peaks were present (Abdallah and Harrad, 2011). This could indicate metabolism of HBCDs to PBCDs which would not be apparent in the soil or dust (Abdallah and Harrad, 2011).

Table 5.1 Concentrations of PBCDs and TBCDs in UK soil samples (pg/g)

Location	PBCD	TBCD
Birmingham (n=2)[a] Summer	<10	<10
Birmingham (n=2)[a] Winter	<10	<10
Edinburgh	<10	<10
Worcester	<10	<10
Aberdeen	<10	<10
Preston	<10	<10
Southampton	80	<10
UEA Campus	<10	140
Rugby	<10	<10
Saffron Walden	310	1300
Brewood	<10	<10
Daventry	10	10
Keele	<10	240
Lancaster	250	<10
Essex	<10	200
Wales (Llandudno)	<10	<10
Cornwall (Helston)	<10	<10
London	7300	1090
Stevenage	150	<10
Bushmills	<10	<10
Norfolk	<10	<10
York	<10	<10
Suffolk	20	<10
Scoat Tarn	<10	<10
MEAN	**342**	**128**
σ_{n-1}	**1484**	**336**
RSD %	**434**	**263**
MIN	**<10**	**<10**
MAX	**7300**	**1300**
5th percentile	**5.0**	**5.0**
95th percentile	**301**	**962**

[a]where more than one sample was analysed, the values given are the average.

For the purposes of statistics the non-detected concentration used assumed to be 0.5 x LOD, where LOD =blank concentration

Figure 5.1: PBCDs in a London soil sample

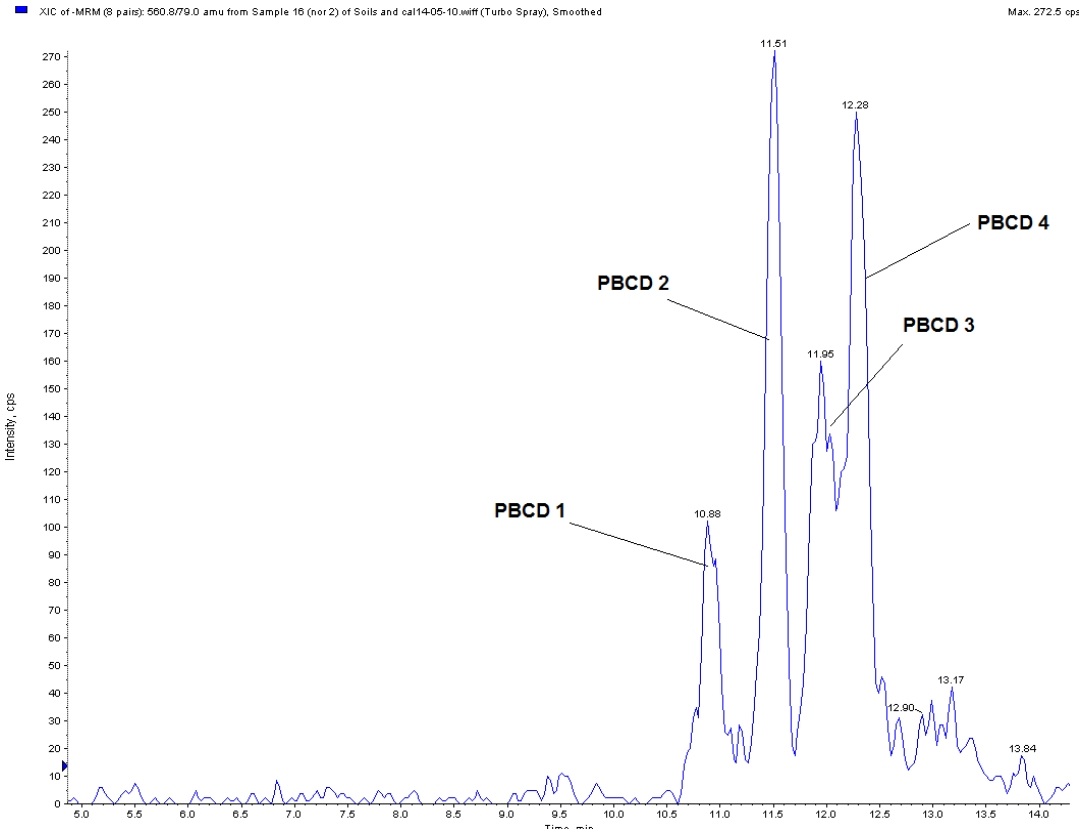


Figure 5. 2: PBCDs in dust (Harrad et al., 2009a)

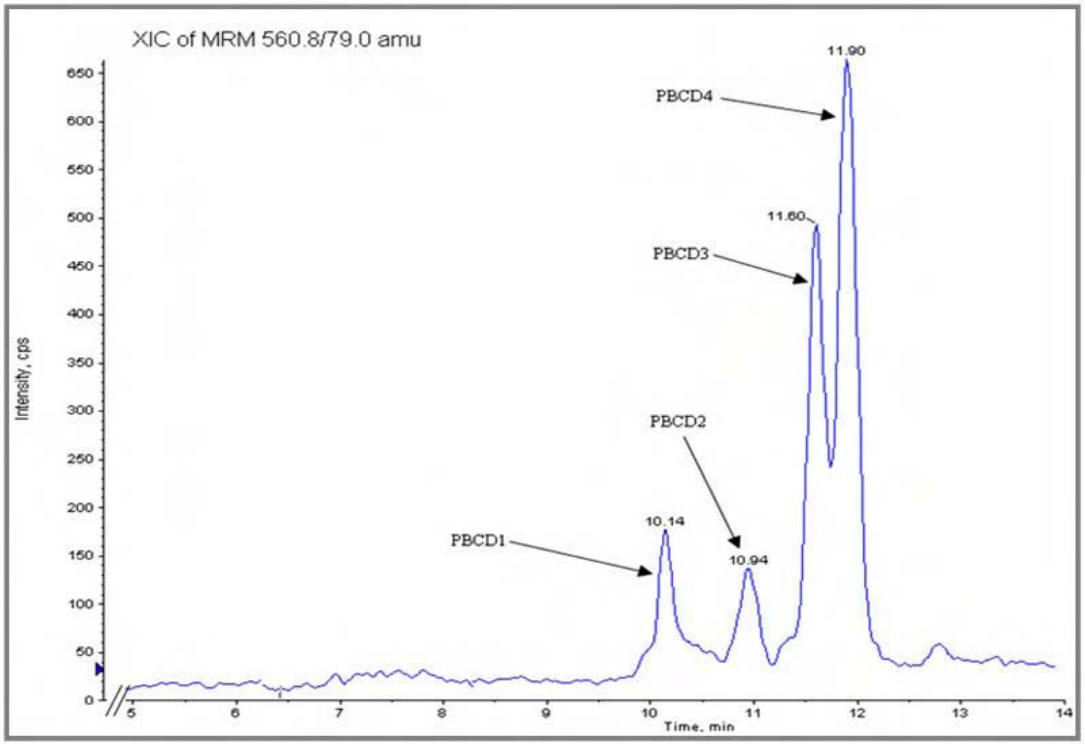


Figure 5.3: chromatograms of (a) PBCDs and (b) TBCDs in human milk (Abdallah and Harrad, 2011)

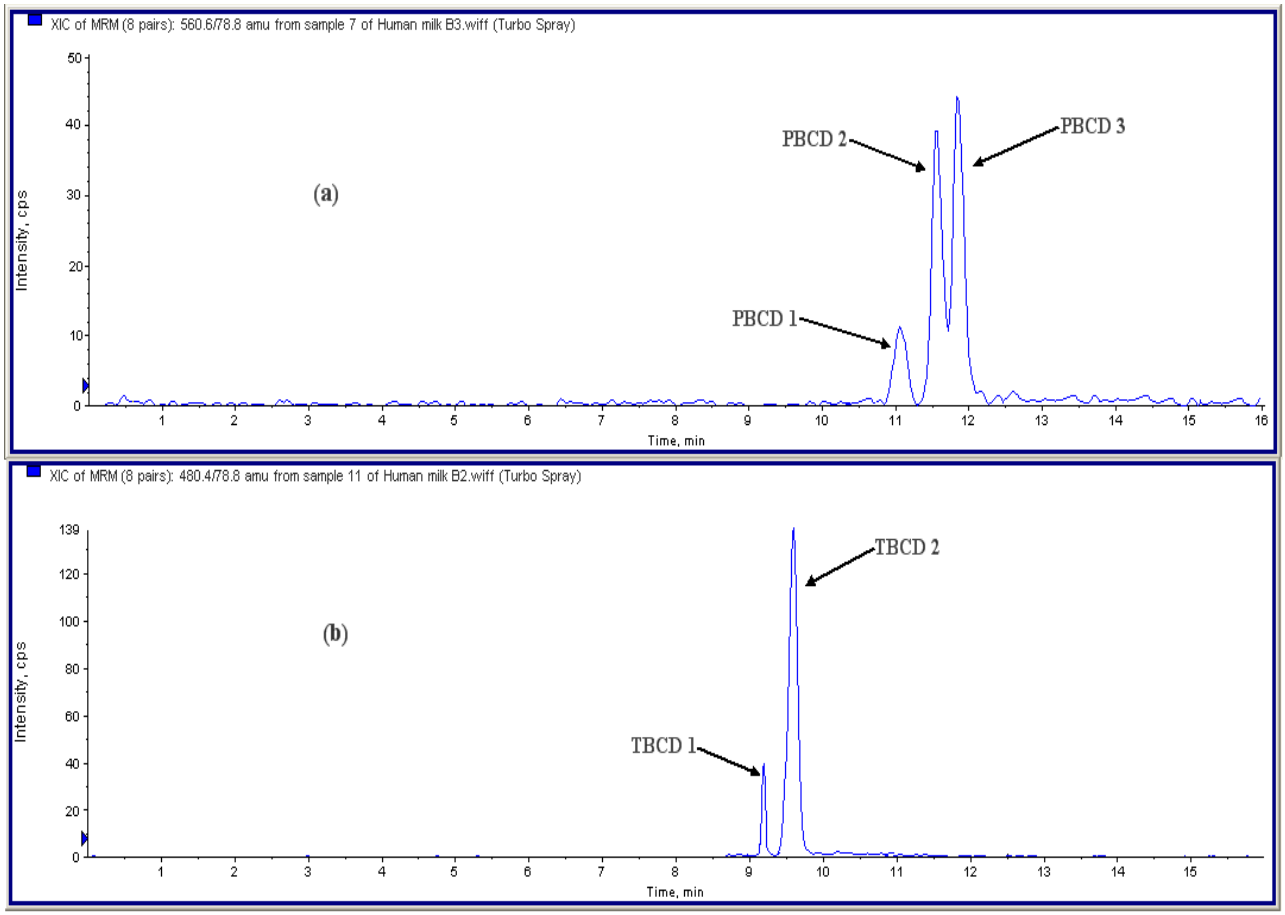


Figure 5.4: TBCD in a London soil sample

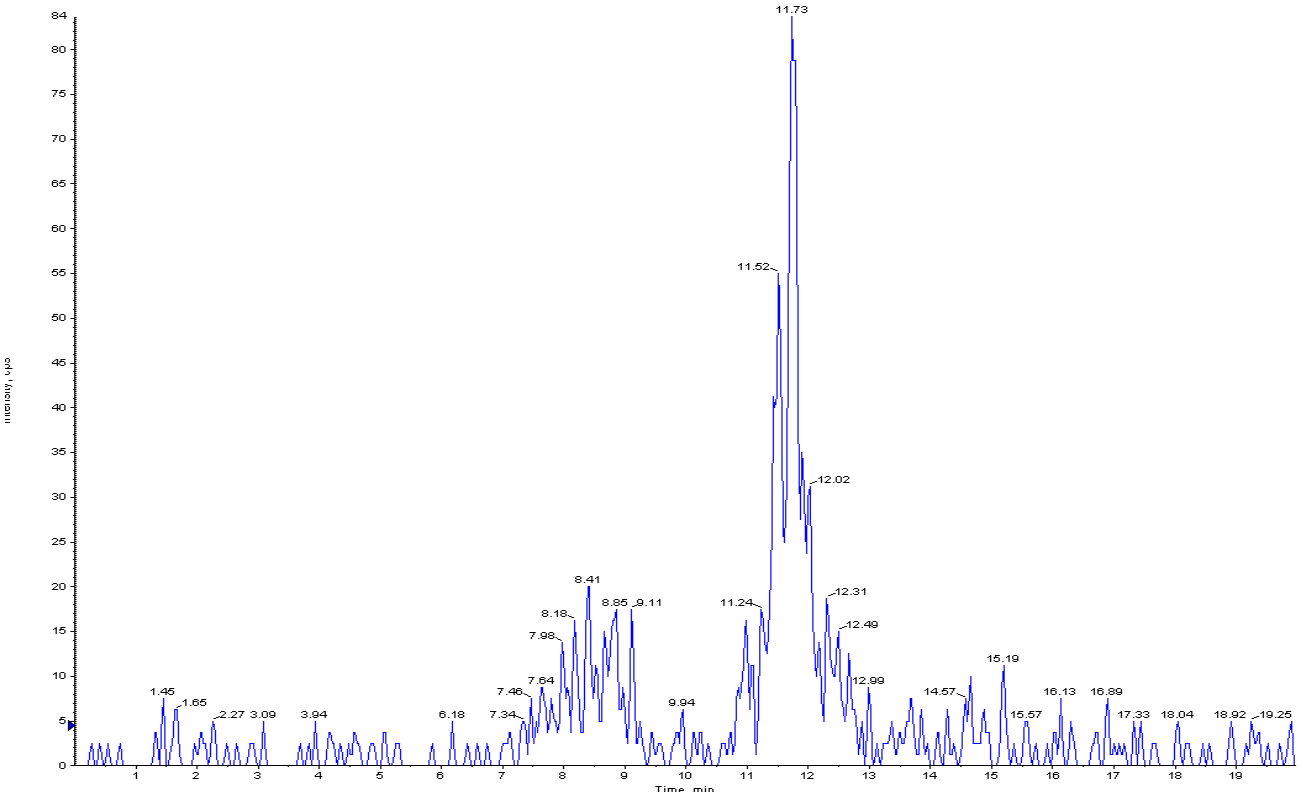


Figure 5.5: TBCD in a dust sample (Abdallah and Harrad 2009)

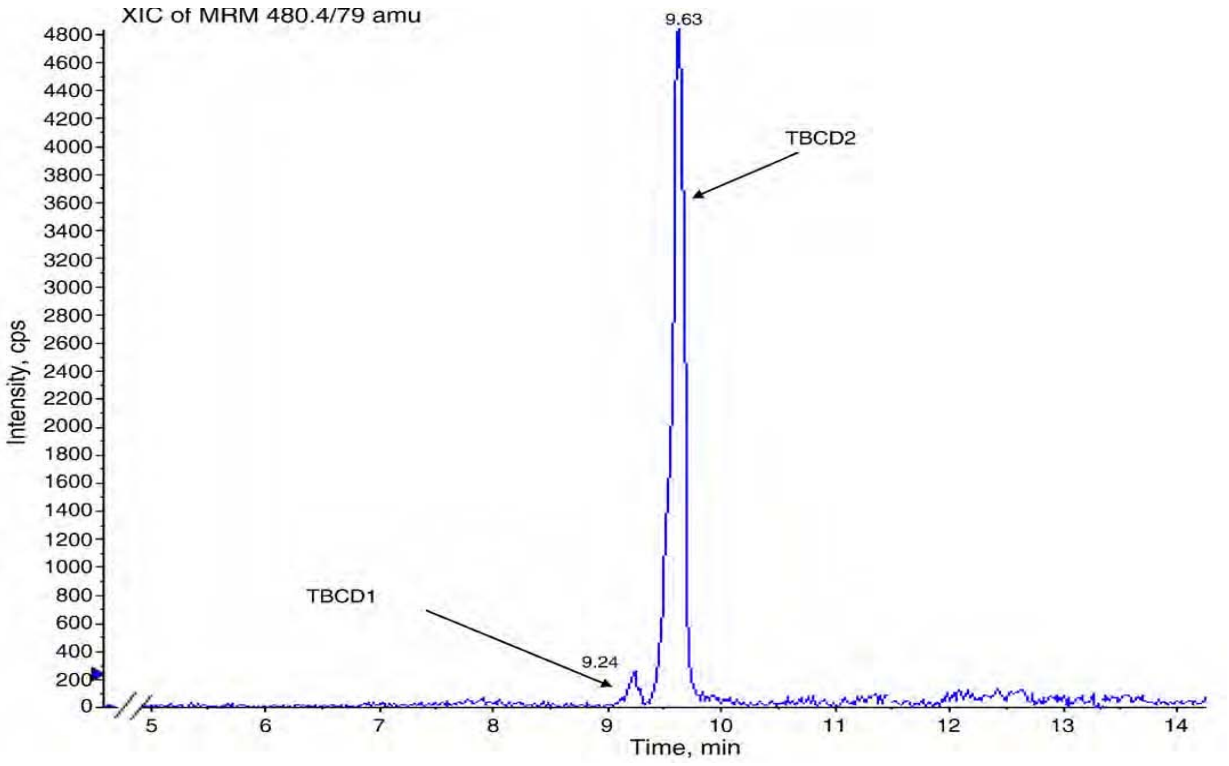


Figure 5.6: PBCD in a technical HBCD formulation

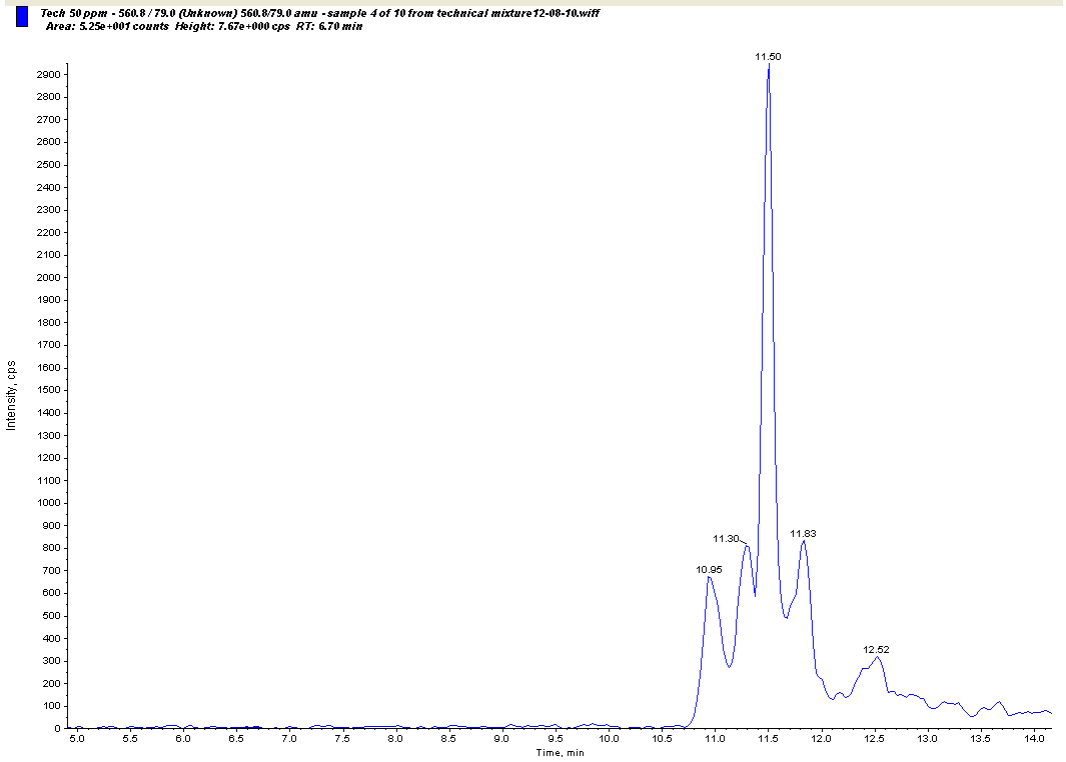
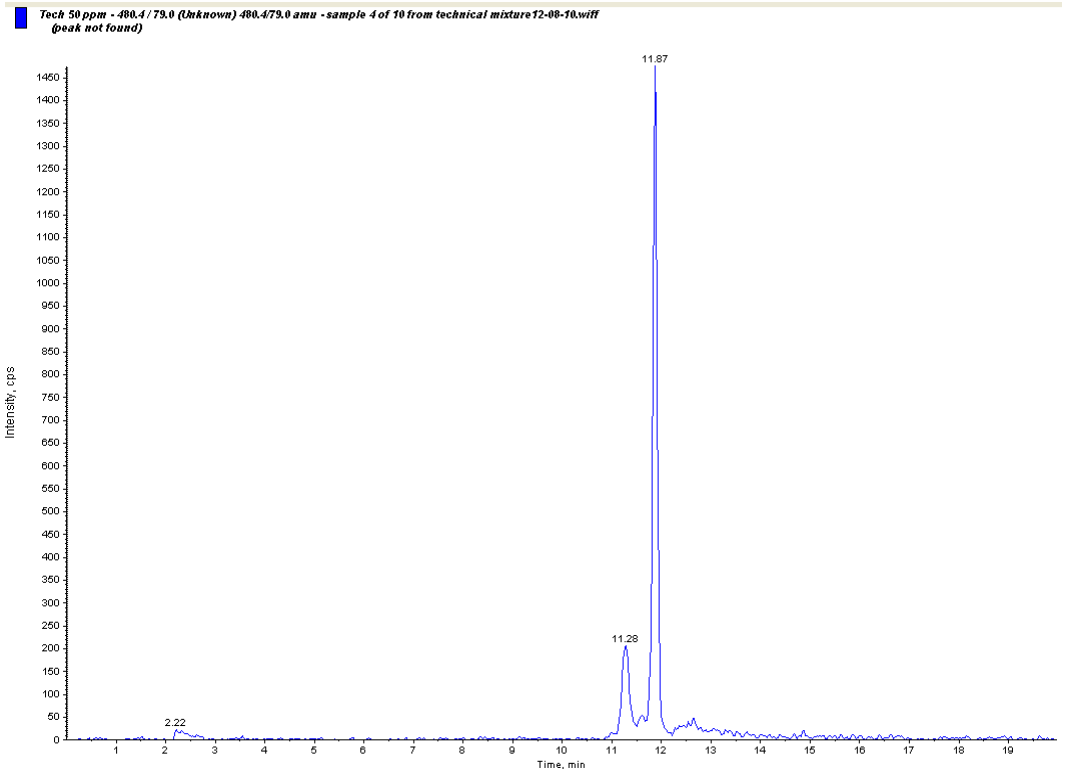


Figure 5.7: TBCDs in a technical HBCD formulation



5.3.2 Degradation products of HBCD in soil from Australia

PBCDs and TBCDs were also determined in the Australian soil samples. In contrast to the UK soil samples, there are no detectable levels of PBCDs in any of the Australian soil samples but TBCDs were measured in the majority of these samples. TBCD concentrations ranged from 2.3-450 pg/g. The degradation products found in Australia were different in pattern to those seen in the UK. Specifically, while TBCDs were detected in nearly all Australian soils, PBCDs were not detected in any. This may be indicative of different degradation pathways, or that the higher temperatures in Australia have "driven" the degradation via sequential dehydrobromination further towards lower brominated products. A greater ratio of degradation products to the parent HBCDs may be expected in the Australian soils compared to their parent compound HBCD as the solar irradiance is higher than the UK. There was no evidence of photolytic transformation of α-HBCD to γ-HBCD in the Australian samples with percentage contributions of all three of the isomers being the same to that seen in the commercial mixture. This may be due to the small number of samples analysed.

Table 5.2 Concentrations of TBCDs in Australian soil samples (pg/g)

	∑TBCD
Currumbin Goldcoast	57
Canberra	2.3
Sydney	3.4
Newcastle	18
Woolongong	<0.2
Launceston	454
Perth Duncraig	<0.2
Perth Kings Park	7.5
Whyalla	249
Port Pirie	77
Port Phillip	68
Kwinana	7.1
Gympie Forestry	27
Lismore	99
Wagga Wagga	4.5
Alice Springs	6.3
Central Tasmania	<0.2
MEAN	64
σ_{n-1}	119
RSD %	187
MIN	<0.2
MAX	454
5th percentile	0.1
95th percentile	290

For the purposes of statistics the non-detected concentration used was 0.5 x LOD

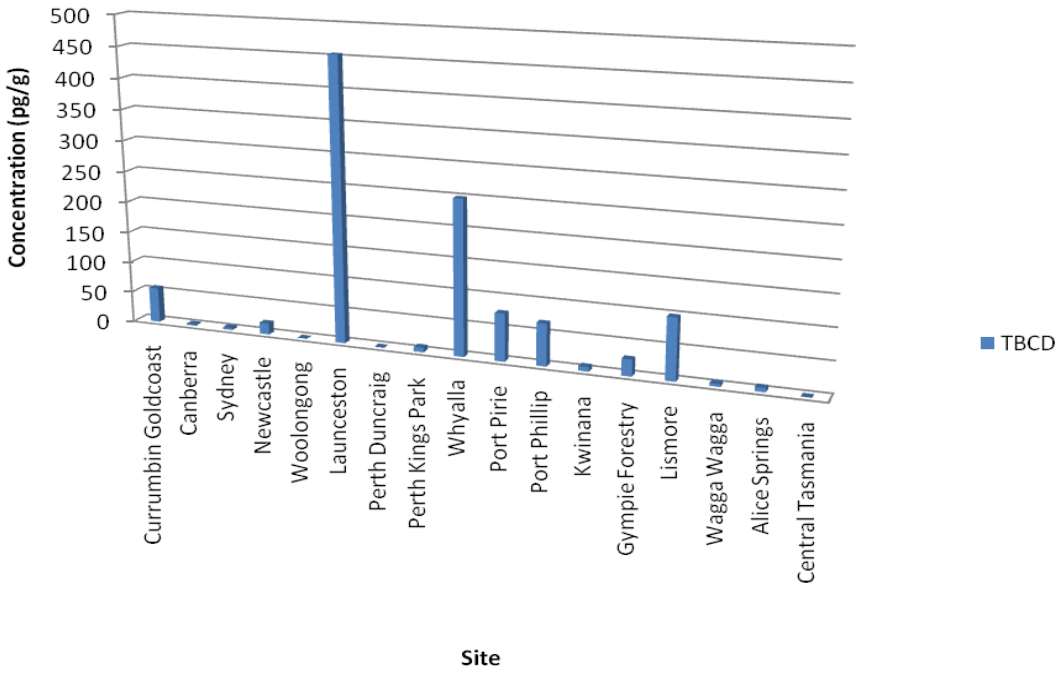


Figure 5.8 TBCD (pg/g) in samples from Australia

5.4 Degradation products in curtain samples

Degradation products were determined in textile samples from Japan where two different types of curtains containing HBCDs were alternately exposed to and shielded from light (Kajiwara and Takigami, 2010). They used 3 black textiles (95-99% polyester) and 3 light blue textiles (polyester). One of each type of textile was shielded from light, one was exposed to light over 371 days, and one sample was covered in aluminium foil and also left in sunlight for 371 days which was used as a dark control sample. They found in this study that there was no marked loss of HBCD diastereoisomers. The results for PBCDs and TBCDs are shown in table 5.3.

Table 5.3 Concentrations of PBCDs and TBCDs in curtain samples

Curtain sample	Curtain treatment	Concentration ng/mg	
		PBCDs	TBCDs
1	Black textile non exposed	368	41
2	Black textile after 371 days of sunlight	297	32
3	Black textile after 371 days of sunlight covered with aluminium foil	336	37
4	Light blue textile non exposed	50	4.1
5	Light blue textile after 371 days of sunlight	22	2.5
6	Light blue textile after 371 days of sunlight covered with aluminium foil	58	4.2

Four isomers of PBCDs were present in the curtain samples, as seen in soils and dust. Interestingly, the light blue curtain which was exposed to light over 371 days contained nearly half the amount PBCDs and TBCDs than the non exposed sample and the covered textile. This could suggest that photolytic degradation is occurring of the PBCDs and the TBCDs to form lower brominated compounds. There was also a small reduction of the degradation products in the exposed black textile although it is not as marked. Kajiwara and Takigami did not observe a loss of the HBCD diasteroisomers in the exposed curtains, which might be expected if photolytic degradation has taken place (Kajiwara and Takigami, 2010). This is in contrast to the study by Harrad et al., who observed a photolytically mediated shift from γ-HBCD to α-HBCD after exposing indoor dust to light over 1 week and a slow degradative loss of HBCD with a decrease in ∑HBCD concentration and an increase in ∑PBCD concentration (Harrad et al., 2009a). They also observed this in standards after exposure to

light and which also showed a small net loss of ∑HBCD via degradation. As the curtain samples contained a very high concentration of HCBD (Light blue textile 43000 mg/kg ∑HBCD and the black textile 42000 mg/kg ∑HBCD) it could be possible that degradation was taking place but at a slow rate which was not discernable at these high concentrations.

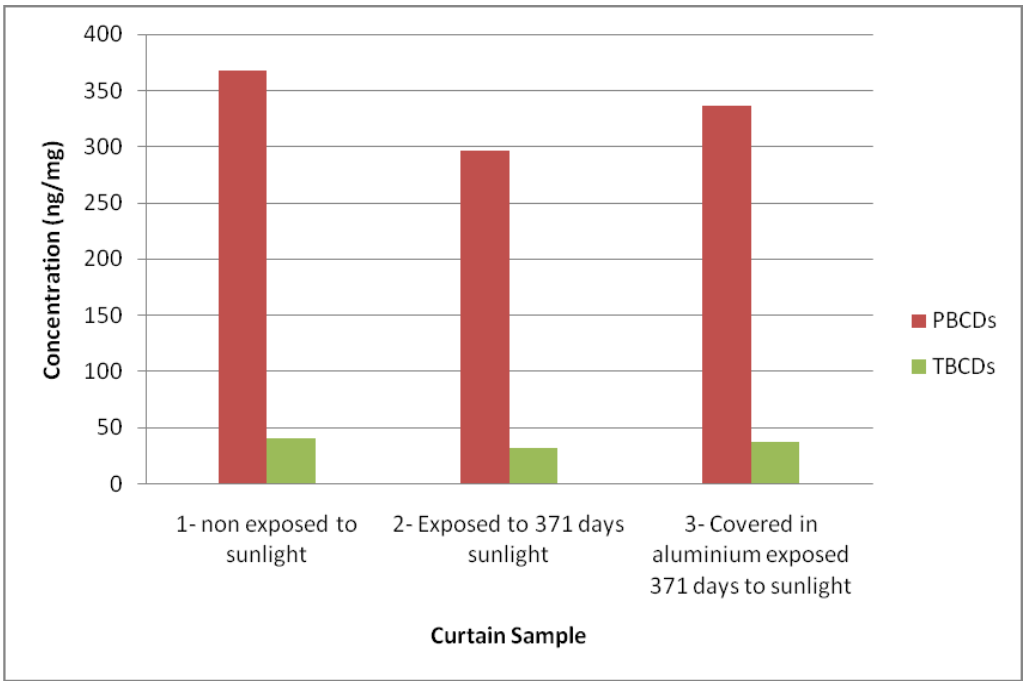


Figure 5.9: Concentrations of PBCDs and TBCDs black curtain samples

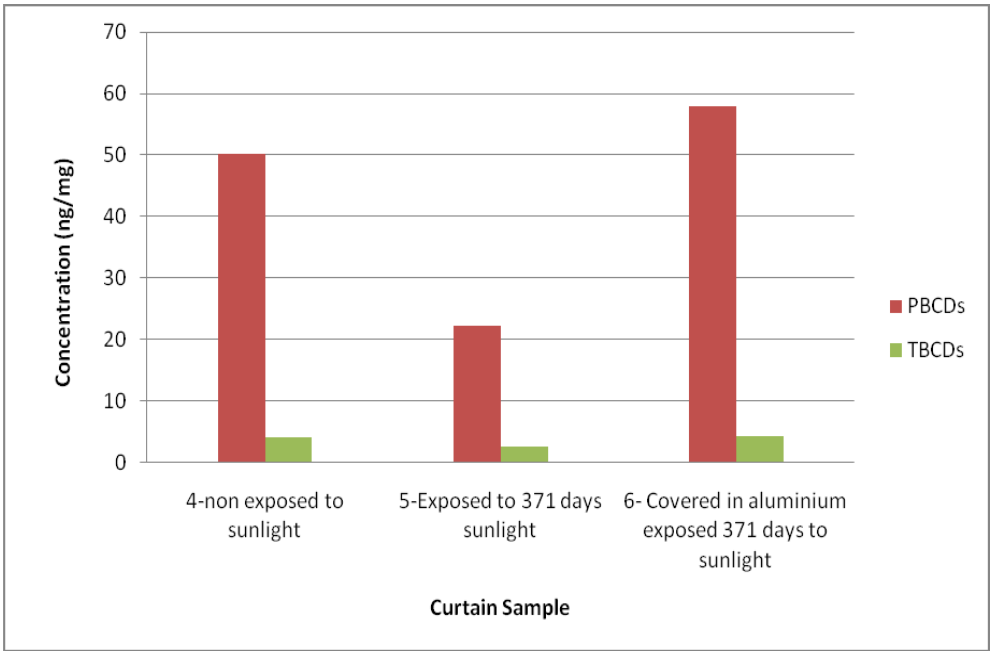


Figure 5.10: Concentrations of PBCDs and TBCDs in light blue curtain samples

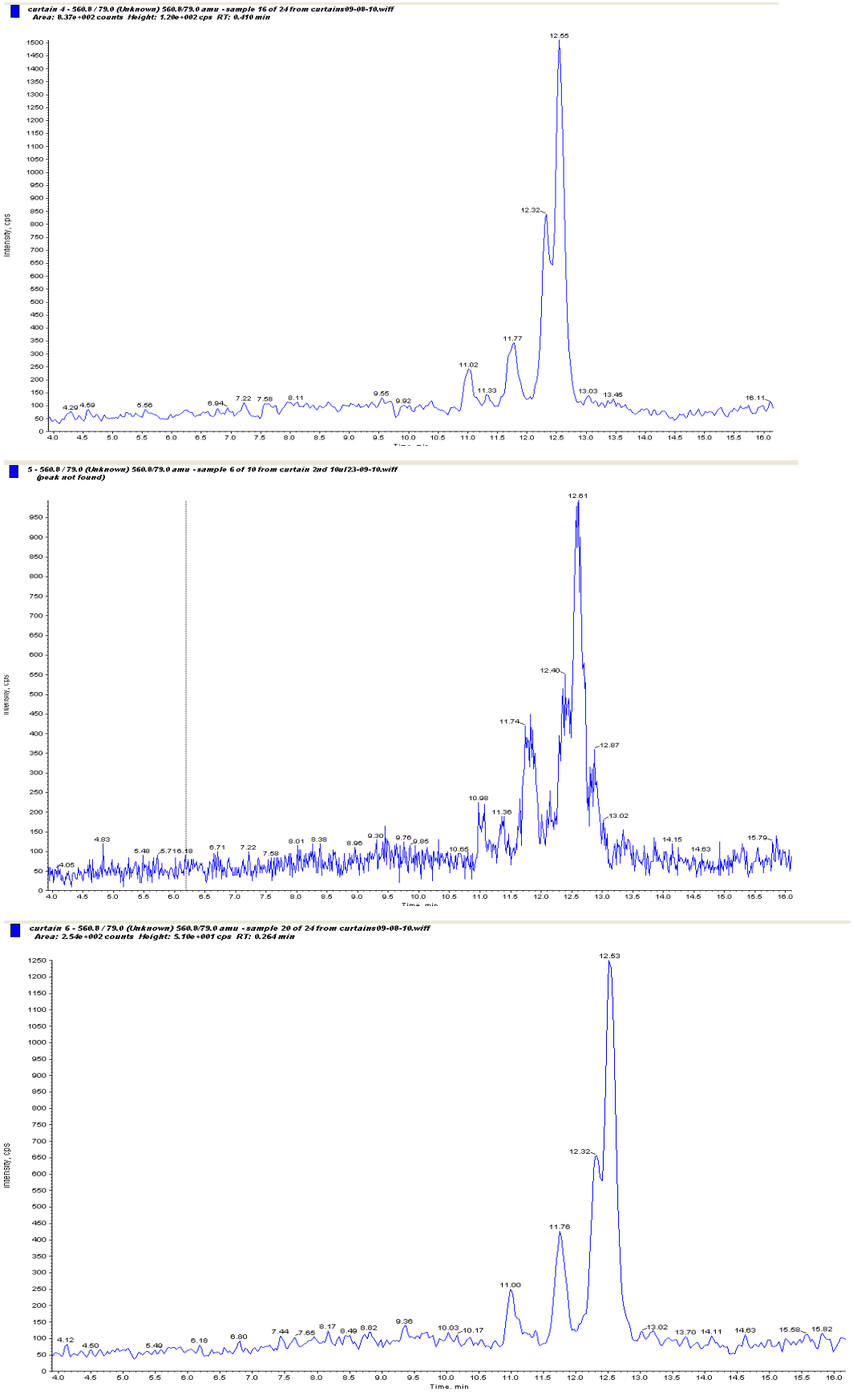


Figure 5.11 PBCDs in curtain samples 4, 5 and 6

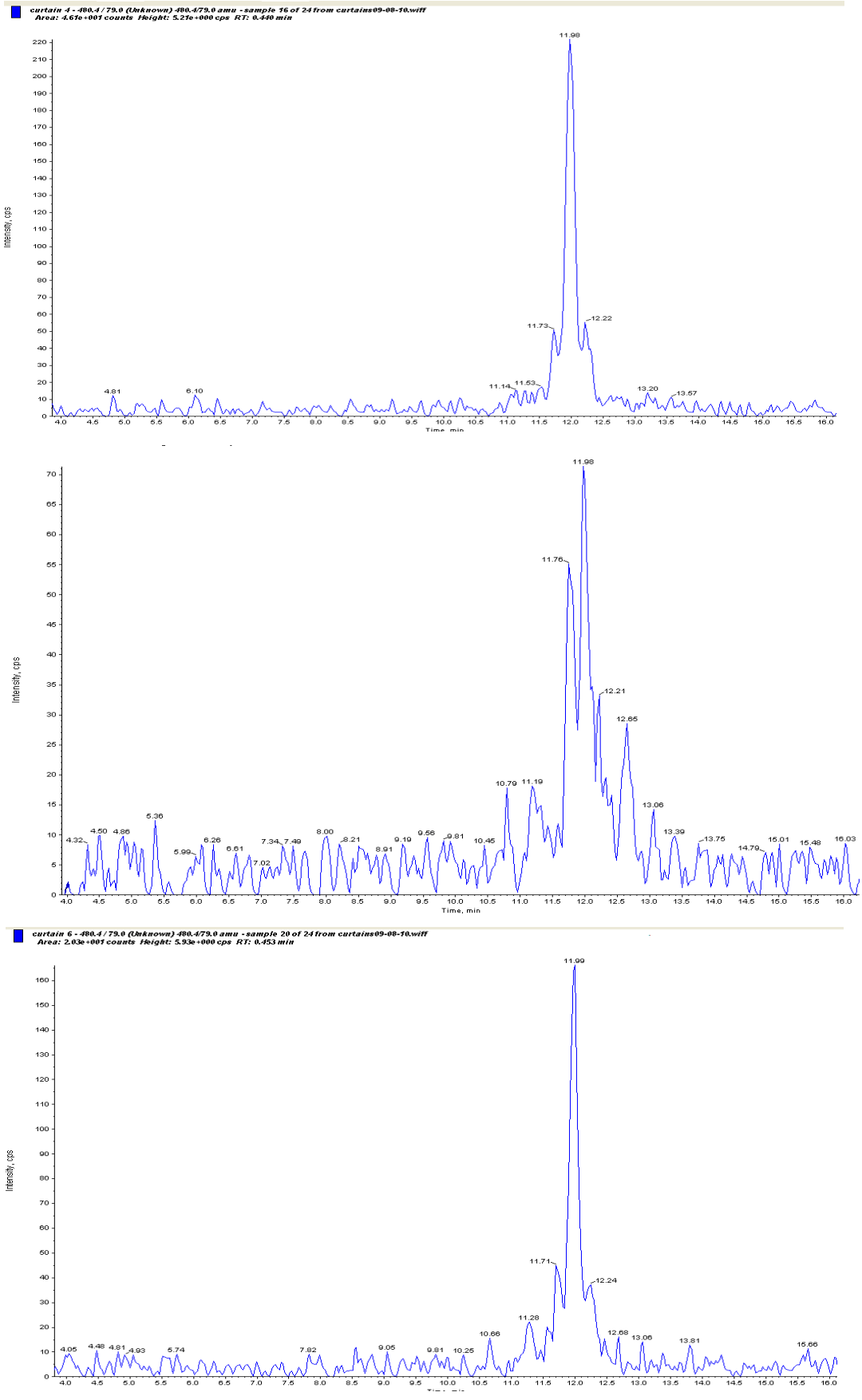


Figure 5.12: TBCDs in curtain samples 4, 5 and 6

5.5 Summary

Two degradation products of HBCD, TBCD and PBCD were semi quantitatively determined in samples of soil from the UK and Australia as well in a technical mixture and curtain samples which had been exposed and shielded from sunlight.

The degradation products TBCDs and PBCDs were determined in 9 of the 23 UK soil samples. This could be an important area of interest as more is discovered about these compounds. This to our knowledge is the only data of its kind of these products in soil samples. These findings suggest that HBCD in soil degrades via the sequential loss of HBr, as seen in dust and sediment samples. PBCD (4 isomers) and TBCDs (2 isomers) were also detected in a technical mixture of HBCD. These degradation products were also found to already be present in the commercial mixture. It is possible therefore that the degradation products are generated via thermal processes during the production of HBCD and its introduction into products which then leach into the environment.

Whereas both PBCDs and TBCDs were detected in soils from the UK, in the Australian soils PBCDs were below the LOD in all samples and TBCD was detected in 14 of the 18 samples. This could indicate that any PBCDs have been further degraded under the warmer and sunnier Australian climate. As the diastereoisomer profiles of HBCD in these samples indicate that photolytic degradation is unlikely to be occurring in these samples it could be suggested that thermal degradation may have resulted in the predominance of TBCD.

In HBCD-treated curtain samples, no discernible differences were observed in levels of PBCDs and TBCDs in black curtain samples which had been exposed to sunlight compared to

those shielded from light. However, significantly lower concentrations of both PBCDs and TBCDs were detected in the light blue curtain sample exposed to sunlight compared to the equivalent samples shielded from light. The curtain results indicate that light could be influencing degradation in the light blue sample; however it is not completely clear from these results. These data show that degradation products PBCDs and TBCDs are present in the curtain samples indicating that HBCD could be degraded via the loss of HBr. However, this may simply be due to the presence of such degradation products in the commercial HBCD formulation. Further more detailed studies are required to determine the factors influencing the formation of PBCDs and TBCDs in environmental samples. Moreover, efforts should be made to determine whether lower brominated degradation products produced by sequential dehydrobromination (or debromination) are also present.

CHAPTER 6: CONCLUSIONS

6.1 Synopsis

The pathways via which POPs distribute throughout the environment is an important area of interest. Although PCBs were banned in the 1970s, existing stocks still continue to pose environmental problems due to their persistence. HBCD is still in use and is a high production brominated flame retardant. There has been growing interest in recent years regarding HBCDs due to their continued use in the environment and limited information on their potential adverse health effects.

The main aim of this thesis was to determine the soil-to-herbage pathways of POPs using chiral signatures. This built upon previous work by Robson and Harrad, and Jamshidi et al., who utilised chiral signatures of PCBs (Robson and Harrad; Jamshidi et al., 2007). In this study PCBs and HBCDs were used as indicator compounds to enhance understanding of these pathways due to their ubiquitous nature in the environment and the fact that some of their isomers are chiral. PCBs have been shown in previous studies to exhibit enantioselective microbial degradation in soils. The secondary aim of this thesis was to determine HBCDs in soils from the UK and Australia. Degradation products of HBCD are also an interesting area of recent research. In this study they were determined in samples of soil, treated textiles and a commercial mixture.

The main conclusions from this thesis are as follows;

1. At the urban site used in this study the concentrations of ∑PCBs in air were found to increase with height from the ground. This indicates that soils are not contributing to the bulk of PCB contamination in background air. These findings also support the idea

that the bulk of PCBs in air are likely to be due from indoor air and not soils (Jamshidi et al., 2007).

2. Volatilisation from soil is a potentially important route of PCBs into grass. Chiral signatures of PCB 95 in air measured at 3 cm from the ground were found to be close to those seen in soils. Chiral signatures in grass were intermediate between those seen in soils and those seen in 'bulk' background air. This highlights an important mechanism by which the substantial reservoir of PCBs in soil could be transferring into the food chain. These findings have important implications for the biogeochemical cycling of PCBs.
3. Concentrations of HBCDs in UK soil are comparable to those seen for PBDEs and PCBs (Harrad and Hunter 2006, Jamshidi et al., 2007). Like PCBs they have become ubiquitous in the environment and are found in most environmental media. A preliminary environmental budget for HBCDs in the UK environment found that soils were the greatest sink for HBCD in the UK environment, and that the total amount of HBCD in the UK environment is of a similar magnitude to that for PCBs in the 1990s.
4. HBCDs were found to be close to racemic in all soils from the UK and Australia from the sites used in this study. Although there are a relatively small number of samples it appears that enantioselective microbial degradation of HBCDs is not occurring in soils. Australian soils were also seen to be racemic implying enantioselective microbial degradation was not affected by the different climatic conditions.
5. The HBCD degradation products PBCD and TBCD were detected in soils from the UK, and TBCD was detected in soil from Australia. The suggested mechanism for degradation of HBCD in soil from the UK is via the sequential loss of HBr.

6.2 Recommendations for further research

There are many areas of interest which have been encompassed in this thesis including determining environmental pathways, utilising chiral signatures and determining levels and trends of POPs in the environment. Within these areas there are gaps in knowledge which include;

1. The importance of the soil to plant pathway as a route of POPs into plants. Further research is needed in to these pathways and the potential route into plants for PCBs as well as other POPs with similar vapour pressures could also be transferring into the food chain in this way.
2. Further data on HBCDs from different environmental compartments including soils and grass will help in the understanding of these compounds and their environmental distribution. This will also allow a more accurate budget of HBCD in the environment to be calculated.
3. The deviation of the relative abundance of the different HBCD diastereoisomers from that observed in the commercial mixture that is present in the environment and biota is an area of interest which is not yet fully understood. It is believed to be a complex combination of processes. The diastereoisomers exhibit different toxicities and it is therefore important to ascertain the levels of the individual isomers and their behaviour in the environment. It is also an area of interest to determine if the transformation between the isomers is occurring predominantly during the process of adding HBCD into products or within the environment.
4. Enantioselective degradation of HBCD is still not fully understood. There is currently more enantioselective data for biota samples than that for other environmental samples such as soils and sediments.

5. Degradation products are a growing area of interest. Although recent studies have begun to shed some light onto these products there is very little knowledge in this area. It is therefore a potentially important area of interest to gain further understanding into the mechanisms via which HBCD degrades. A controlled light experiment on a pure HBCD standard should be carried to determine if photolytic degradation is occurring within the UV light range and whether PBCD and TBCD are products. PBCDs and TBCDs are just two potential degradation products that could be formed from HBCD and other lower brominated compounds and the mechanisms by which they formed are also an area for future research. The development of standards for these compounds will be important in gaining further understanding of them and obtaining more accurate data. PBCDs and TBCDs have currently been found in dust, sediments, human milk and from this study in soils. Therefore more knowledge is needed on the potential health effects of these compounds.

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APPENDIX A: Publications

- S. Harrad, E. Goosey, J. Desborough, M. A-E. Abdallah, L. Roosens, A. Covaci. "Dust from UK primary school classrooms and daycare centers: The significance of dust as a pathway of exposure of young UK children to Brominated Flame Retardants and Polychlorinated Biphenyls", *Environmental Science and Technology* 44, 4198–4202 (2010).
- S. Harrad, J. Desborough,, M. A-E. Abdallah. "An overview of contamination of the UK environment with HBCD and its degradation products", *Organohalogen Compounds,* 72, 193-196 (2010).
- Desborough, J. and S. Harrad (2011). "Chiral Signatures Show Volatilization from Soil Contributes to Polychlorinated Biphenyls in Grass." Environmental Science & Technology **45**(17): 7354-7357.

APPENDIX B: Data tables

Table 1: Concentrations (pg g^{-1} dry weight) and Enantiomer Fractions of PCBs in Soil Samples in 2009 and 2010 Campaigns

Sampling day	**Enantiomer fractions**		**pg/g dry weight**							
2009	**PCB 95**	**PCB 36**	**PCB 28+31**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 180**	**∑PCB**
15	0.445	0.524	12.98	10.66	27.42	41.85	77.33	92.73	14.94	**278**
29	0.458	0.521	19.86	17.02	30.97	47.68	68.93	99.78	14.84	**299**
44	0.456	0.517	10.27	6.43	14.99	23.84	42.69	54.30	5.92	**158**
58	0.443	<dl	11.14	6.99	18.39	25.12	45.51	50.99	9.17	**167**
72	0.450	0.510	9.72	12.89	27.63	39.44	65.95	86.66	9.86	**252**
85	0.453	0.516	26.39	10.66	22.73	38.98	63.49	78.54	5.09	**246**
100	0.462	0.512	19.50	70.86	136.81	136.42	181.77	238.37	29.72	**813**
114	0.448	0.526	13.89	12.96	25.64	35.62	73.35	95.69	11.82	**269**
2010			**PCB 28+31**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 180**	**∑PCB**
14	0.440	0.512	26.70	24.39	77.34	107.37	206.25	249.37	84.70	**776**
28	0.439	0.505	32.86	32.51	88.23	107.73	234.77	279.30	93.69	**869**
42	0.456	0.502	44.61	35.64	106.11	144.64	321.19	390.02	149.54	**1192**
56	0.445	0.507	42.62	35.18	118.01	149.57	302.33	375.17	137.26	**1160**
70	0.454	0.516	42.36	38.16	133.49	161.02	308.67	410.66	134.21	**1229**
84	0.446	0.501	29.71	23.72	96.03	122.69	258.13	295.13	154.88	**980**

Table 2: Concentrations (pg g^{-1} dry weight) and Enantiomer Fractions of PCBs in Grass Samples in 2009 and 2010 Campaigns

Sampling day	**Enantiomer fractions**		**pg/g dry weight**							
2009	**PCB 95**	**PCB 36**	**PCB 28+31**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 180**	**∑PCB**
15	0.462	0.520	180.56	137.36	118.36	106.76	191.03	165.92	69.19	**969**
29	0.466	0.531	141.31	147.10	127.96	107.63	159.59	140.54	34.21	**858**
44	0.467	0.502	170.83	161.22	129.29	116.62	216.88	178.52	82.55	**1056**
58	0.463	0.507	141.66	181.28	113.06	98.58	215.83	160.33	84.04	**995**
72	0.475	0.515	165.20	156.02	111.01	123.85	227.71	180.16	98.89	**1063**
85	0.474	0.516	299.65	188.32	165.27	136.16	325.66	238.35	138.54	**1492**
100	0.467	0.528	808.14	252.73	183.60	151.71	308.35	240.14	126.71	**2071**
114	0.469	0.519	346.84	191.69	159.37	156.61	333.71	312.25	112.71	**1613**
2010			**PCB 28+31**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 180**	**∑PCB**
14	0.482	0.515	479.86	590.97	421.72	130.01	294.68	169.90	66.84	**2154**
28	0.479	0.517	489.91	454.56	270.98	121.42	244.09	140.59	55.37	**1777**
42	0.465	0.521	335.60	361.55	166.85	74.55	131.57	96.27	31.25	**1198**
56	0.487	0.518	713.55	984.42	716.34	242.60	587.76	331.29	143.82	**3720**
70	0.494	0.493	747.14	1019.87	693.49	220.18	515.24	292.96	98.34	**3587**
84	0.494	0.501	552.81	783.62	540.70	190.86	505.74	285.75	145.45	**3005**

Table 3: Concentrations (pg g^{-1} dry weight) and Enantiomer Fractions of PCBs in Air Samples at different heights in 2009 Campaign

Sampling day (height)	Enantiomer fractions		pg/g dry weight							
	PCB 95	PCB 36	PCB 28+31	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	∑PCB
15 (3 cm)	0.459	**0.517**	-	-	-	-	-	-	-	**-**
15 (10 cm)	0.500	**0.505**	52.23	23.97	9.00	3.09	3.85	2.67	0.58	**95.40**
15 (40 cm)	0.497	**0.505**	97.22	36.60	15.73	6.85	8.26	7.69	1.48	**173.82**
15 (90 cm)	0.503	**0.505**	91.48	31.25	14.97	5.37	7.24	6.22	1.78	**158.30**
15 (130 cm)	0.506	**0.501**	96.60	35.84	18.57	7.77	9.95	7.78	1.76	**178.28**
29 (3 cm)	nd	**0.513**	-	-	-	-	-	-	-	**-**
29 (10 cm)	0.507	**0.503**	52.40	20.93	8.68	3.65	4.00	3.71	0.55	**93.91**
29 (40 cm)	0.512	**0.505**	91.02	35.61	17.53	7.36	7.54	7.01	1.48	**167.56**
29 (90 cm)	0.498	**0.508**	105.12	38.32	21.50	8.83	9.68	9.32	1.78	**194.55**
29 (130 cm)	0.497	**0.510**	113.17	41.17	21.20	9.36	11.31	9.22	1.63	**207.05**
44 (3 cm)	0.449	**nd**	-	-	-	-	-	-	-	**-**
44 (10 cm)	0.501	**0.502**	41.05	19.55	10.70	2.67	5.31	2.28	1.13	**82.68**
44 (40 cm)	0.496	**0.503**	61.32	27.54	18.76	5.09	10.09	4.23	2.40	**129.41**
44 (90 cm)	0.501	**0.503**	64.58	30.50	19.82	5.87	10.92	4.94	2.47	**139.11**
44 (130 cm)	0.502	**0.501**	73.89	33.92	21.57	5.66	12.94	6.59	3.16	**157.75**

58 (3 cm)	nd	**nd**	-	-	-	-	-	-	-	**-**
58 (10 cm)	0.504	**0.494**	31.52	26.50	9.55	2.66	6.08	2.98	1.91	**81.19**
58 (40 cm)	0.496	**0.516**	51.55	35.95	17.34	5.62	9.79	6.00	3.19	**129.45**
58 (90 cm)	0.491	**0.505**	49.97	36.23	18.62	5.69	10.74	6.50	3.66	**131.40**
58 (130 cm)	0.502	**0.512**	71.23	46.02	24.28	7.50	15.02	9.13	5.20	**178.39**
72 (3 cm)	0.447	**0.517**	-	-	-	-	-	-	-	**-**
72 (10 cm)	0.499	**0.510**	33.06	30.13	11.03	2.94	6.05	4.51	3.46	**91.19**
72 (40 cm)	0.496	**0.505**	49.74	34.52	17.82	6.01	11.25	6.63	2.87	**128.85**
72 (90 cm)	0.493	**0.506**	69.72	42.19	22.23	6.70	12.52	8.19	3.19	**164.75**
72 (130 cm)	0.508	**0.501**	70.29	38.90	23.00	6.77	13.93	8.68	3.55	**165.13**
85 (3 cm)	0.451	**nd**	-	-	-	-	-	-	-	**-**
85 (10 cm)	0.506	**0.498**	38.57	25.78	10.89	3.22	6.92	3.99	1.83	**91.19**
85 (40 cm)	0.494	**0.503**	59.55	37.52	21.87	6.80	14.07	8.21	4.01	**152.03**
85 (90 cm)	0.499	**0.507**	65.03	40.74	23.53	7.12	15.50	9.07	4.04	**165.01**
85 (130 cm)	0.500	**0.500**	70.19	40.43	26.61	8.06	18.90	10.41	4.84	**179.44**
100 (3 cm)	0.450	**nd**	-	-	-	-	-	-	-	**-**

100 (10 cm)	0.486	**0.507**	50.55	16.61	9.27	3.39	5.43	3.21	0.84	**89.30**
100 (40 cm)	0.496	**0.499**	115.06	35.17	18.01	6.97	12.22	7.83	1.89	**197.15**
100 (90 cm)	0.496	**0.501**	142.60	39.71	22.00	7.35	14.13	8.58	1.46	**235.83**
100 (130 cm)	0.497	**0.507**	143.66	42.16	24.51	7.90	16.42	9.38	2.63	**246.66**
114 (3 cm)	0.459	**nd**	-	-	-	-	-	-	-	**-**
114 (10 cm)	0.499	**0.505**	41.84	13.53	10.02	2.79	3.98	3.26	0.59	**76.01**
114 (40 cm)	0.498	**0.506**	75.00	24.74	21.58	6.63	9.51	6.84	1.39	**145.70**
114 (90 cm)	0.502	**0.510**	84.80	32.42	24.25	9.29	11.53	9.00	1.50	**172.79**
114 (130 cm)	0.498	**0.497**	98.20	36.84	31.31	11.86	14.27	12.24	1.79	**206.51**

Table 4: Concentrations (pg g^{-1} dry weight) and Enantiomer Fractions of PCBs in Air Samples at different heights in 2010 Campaign

Sampling day (height)	Enantiomer fractions		pg/g dry weight							
	PCB 95	PCB 36	PCB 28+31	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	∑PCB
14 (3 cm)	**0.467**	**0.515**	-	-	-	-	-	-	-	**-**
14 (10 cm)	**0.489**	**0.508**	30.51	21.60	5.74	2.23	1.84	2.29	0.23	**64.44**
14 (40 cm)	**0.499**	**0.502**	46.73	29.81	9.97	5.48	5.15	5.18	0.35	**102.67**
14 (90 cm)	**0.504**	**0.506**	70.54	47.81	14.44	5.46	7.69	6.61	0.18	**152.73**
14 (130 cm)	**0.501**	**0.500**	60.21	38.03	12.06	5.12	6.33	5.46	0.29	**127.50**
28 (3 cm)	**0.468**	**0.518**	-	-	-	-	-	-	-	**-**
28 (10 cm)	**0.488**	**0.508**	37.13	22.71	7.24	3.66	3.14	4.20	<dl	**78.08**
28 (40 cm)	**0.498**	**0.503**	38.96	22.33	8.77	2.61	5.63	2.85	<dl	**81.15**
28 (90 cm)	**0.501**	**0.497**	54.98	30.11	14.14	3.72	8.41	4.56	1.21	**117.12**
28 (130 cm)	**0.501**	**0.508**	63.54	33.40	15.16	5.62	9.40	4.99	1.32	**133.43**
42 (3 cm)	**nd**	**nd**	-	-	-	-	-	-	-	**-**
42 (10 cm)	**nd**	**nd**	26.67	12.22	3.53	0.81	0.48	1.13	<dl	**44.83**
42 (40 cm)	**0.501**	**nd**	36.72	16.73	5.90	2.14	2.38	2.29	<dl	**66.16**
42 (90 cm)	**0.499**	**0.504**	57.31	26.54	10.43	3.75	5.78	4.92	0.82	**109.57**

42 (130 cm)	**0.501**	**0.502**	60.00	26.49	11.09	4.36	6.63	4.98	0.94	**114.48**
56 (3 cm)	**0.470**	**nd**	-	-	-	-	-	-	-	**-**
56 (10 cm)	**0.490**	**nd**	31.28	14.69	5.77	1.66	0.87	0.61	0.04	**54.93**
56 (40 cm)	**0.500**	**0.502**	39.90	21.85	9.19	4.19	4.93	3.49	1.16	**84.72**
56 (90 cm)	**0.500**	**0.497**	65.72	39.02	22.37	9.20	11.72	10.98	1.91	**160.92**
56 (130 cm)	**0.499**	**0.502**	70.60	45.69	25.99	11.21	14.26	12.99	3.63	**184.37**
70 (3 cm)	**0.468**	**nd**								
70 (10 cm)	**0.489**	**0.500**	41.18	16.74	6.88	1.97	3.35	1.63	0.03	**71.78**
70 (40 cm)	**0.499**	**0.503**	50.63	23.42	7.82	3.19	3.83	1.97	0.70	**91.55**
70 (90 cm)	**0.499**	**0.506**	79.53	34.42	17.23	6.18	9.47	6.36	1.58	**154.77**
70 (130 cm)	**0.505**	**0.503**	89.12	42.40	19.87	6.29	11.36	8.18	4.05	**181.26**
84 (3 cm)	**nd**	**nd**	-	-	-	-	-	-	-	**-**
84 (10 cm)	**0.489**	**nd**	27.58	11.78	3.87	1.67	2.04	1.29	<dl	**48.22**
84 (40 cm)	**0.495**	**0.508**	29.71	12.84	4.12	1.74	3.11	1.76	1.73	**55.02**
84 (90 cm)	**0.501**	**0.502**	50.75	21.79	10.34	3.68	7.47	5.36	2.81	**102.19**
84 (130 cm)	**0.504**	**0.495**	64.33	30.12	15.06	5.97	12.38	7.62	4.83	**140.31**