PCB Chiral Signatures in Grass, Soil, and Air: Soil-to-Grass Transfer Is More Important than Realised

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Introduction

Polychlorinated biphenyls (PCBs) are organochlorine chemicals that found widespread use in a diverse range of applications,with around 1.2 million tonnes produced worldwide[1]. Owing to their toxicity, their production - but not their use - ceased throughout most of the industrialised world in the late 1970s. Although UK human exposure to PCBs has fallen in recent years in response to the cessation of their production in the late 1970s[2], human health concerns remain. The dietary exposure pathway is significant, with transfer *via* the soil/air-grass-meat/milk food chains featuring prominently. As a result, furthering understanding of the mechanisms of how PCBs enter grass is a research priority. Although there is a substantial body of research that has *indirectly* suggested that PCBs enter grass predominantly *via* uptake from air, rather than from soil (either *via* root-to-foliage translocation, or foliar contamination with either soil particles or PCBs volatilising from soil)[3], *direct* evidence has hitherto been lacking.

This paper reports chiral signatures of PCB#s 95 and 149 in grass and compares them with those in soil and outdoor air samples at the same location. In summary, the relationship between signatures in grass and those in soil and air should afford *direct* insights as to the relative significance of uptake of PCBs from soil and air into grass. Note that although we attempted to measure PCB#136, only a very small number of samples displayed chiral signatures that met the minimum signal to noise ratio quantification criterion, and these data are not reported here.

Materials and Methods

Chiral signatures – expressed as enantiomeric excess (%ee) - of PCB#s 95 and 149 were determined in previously stored extracts of grass. The 2 experiments from which samples were taken consisted of: (a) a "kinetic" experiment conducted between May and July 2000 that measured the influence of exposure time of uptake of PCBs by grass[4]; and (b) a "seasonal" experiment in which samples of grass were harvested at roughly monthly periods between March 1999 and February 2000. In the "kinetic" experiment, a plot of pasture grass divided into 6 adjacent 1 m^2 sub-plots was "fenced-off" within 2 m of the Elms Road Observatory site (EROS) in Birmingham, UK where measurement of chiral signatures of PCBs in outdoor air and soil has been previously reported[5]. The plot was cut back on 5th May 2000, sampled after 14 days, and then at 32, 42, 55, 70, and 83 days. The species composition of the mixed sward was typical of U.K. pasture grassland. In the "seasonal" experiment, a separate (i.e. distinct from those used in the "kinetic" experiment) 1 m^2 plot of pasture grass (of similar species composition to that present in the "kinetic" experiment) within 2 m of EROS was harvested at approximately monthly intervals. Each sample reported here consists of the grass harvested from the same plot over each period shown in Table 1.

Sample preparation, extraction and purification

Following harvesting grass samples (30 – 100 g fresh weight, depending on plot yield) were washed carefully with distilled water to remove adhering soil, frozen by immersion in liquid nitrogen and dried by grinding with a similar mass of anhydrous sodium sulfate (also frozen with liquid nitrogen). Subsequent extraction and purification prior to GC/MS analysis was as reported elsewhere[6].

Enantioselective GC/MS

All extracts were subjected to enantioselective GC/MS according to our previously validated protocol[5] on a Fisons' MD-800 instrument operated in SIM mode and fitted with a Chirasil Dex (10% permethylated 2,3,6-tri-*O*-methyl *b*-cyclodextrin as chiral selector on a polysiloxane backbone, 25m x 0.25mm x 0.25µm, Chrompack) column.

Results and Discussion

Table 1 shows the %ee values of PCB#s 95 and 149 in grass samples harvested during both the "kinetic" and "seasonal" experiments and compares them with those reported previously in outdoor air and topsoil at the same site – albeit over a different time period[5]. While the signatures in outdoor air are racemic (defined as %ee = 0±1%), those in topsoil and most grass samples are not. Comparison of chiral signatures provides *direct* evidence that soil-to-grass transfer may be far more important than hitherto realised. Figures 1a and 1b illustrate the variation of the absolute value of %ee in grass samples with exposure time in the "kinetic" experiment. There is a general – though not entirely conclusive - deviation from the racemic signatures present in outdoor air (sampled at a height of 1.5 m[5]) towards the non-racemic signatures observed in soil at this site with increasing exposure time. Figures 2a and 2b show the variation in chiral signatures over the course of a 12 month period during the "seasonal" experiment. Here, there is a clear pattern; starting from racemic signatures in March (the start of the growing season in the U.K.), the signatures become increasingly non-racemic (approaching those observed in topsoil at the same location) throughout the growing season (i.e. to end October), before a gradual return to near-racemic signatures by the end of winter (February). There are several possible explanations for the above observations, namely:

(a) the signatures in grass are as a result of direct contamination with soil. We believe this to be unlikely, given that: (i) all grass samples were carefully washed with distilled water prior to freeze-drying; and (ii) if adhering soil particles were present, it is likely that their influence would be random, and neither seasonal, nor dependent on exposure time.

(b) the signatures in grass are produced by within-plant enantioselective degradation. Again, although impossible to rule out completely, this seems unlikely given the rate at which such degradation would have to occur to bring about the observed pattern in the "kinetic" experiment.

(c) the signatures in grass arise due to root uptake and translocation of soil-borne contamination.

(d) the signatures in grass are due to volatilisation from soil and subsequent gas phase deposition.

From the data presented here, it is not possible to distinguish accurately the relative contributions that processes (c) and (d) make. However, (c) has been shown to be negligible for grass[7]. Instead, although an earlier study at EROS demonstrated volatilisation from topsoil to exert a negligible influence on chiral signatures in outdoor air sampled at 1.5 m above the soil surface[5]; we believe (d) provides the most likely explanation of the observed chiral signatures in grass for the following reasons:

1. as we have stated previously[5], it is possible that PCBs are volatilizing from soil at EROS, but that they only exert an impact on chiral signatures in air very close to the soil surface. Although our earlier study could not detect such an attenuation of the impact of volatilization from soil relative to the influence of advective transport with height above the soil surface (presumably because of the low concentrations in soil at EROS – 51 pg g^{-1} of PCB #95 and 107 pg g^{-1} for PCB 149[5]); such observations have been reported previously for atmospheric chiral signatures of *a*-HCH[8], and for atmospheric concentrations of SPCB[9], where concentrations in soil have been far higher (42 ng g^{-1} *a*-HCH, and between 1.1 and 635 mg g^{-1}SPCB).

2. it is consistent with the observed pattern (see Figures 2a and 2b) in the "seasonal" experiment. In essence, the signature in grass most closely resembles that in topsoil during the warmer months. Indeed, for PCB# 95 (but not PCB# 149), there is a statistically significant positive relationship ($p<0.05$) between [%ee] in a grass sample and the air temperature at EROS averaged over the sampling period.

3. it is consistent with the predictions of the mathematical model developed by Trapp and Matthies[10] that uptake by grass of 2,3,7,8-TCDD volatilised from soil was possible – albeit restricted to only the lowest few centimetres of foliage.

References:

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Table 1: %ee Values for PCBs# 95 and 149 in Grass, Soil[a], and Outdoor Air[a] Samples at EROS

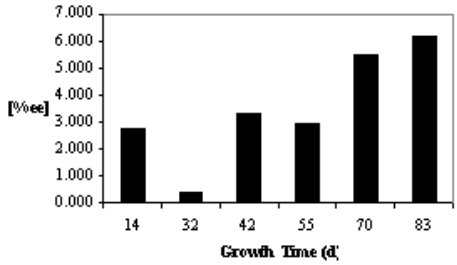
Sample	%ee of PCB 95	%ee of PCB 149
K14[b]	-2.731	Not quantifiable
K32	-0.379	-0.047
K42	-3.285	3.951
K55	-2.943	6.13
K70	-5.511	6.577
K83	-6.181	1.496
S21/12-25/1[c]	-4.347	4.266
S16/1-20/2	-1.565	Not quantifiable
S21/2-30/3	-0.328	2.056
S31/3-4/5	-4.255	2.693
S28/8-28/9	-11.12	7.313
S29/9-2/11	-11.39	7.249
S3/11-19/11	-8.295	6.469
S20/11-20/12	-5.968	4.266
Outdoor Air (n=32)	-0.791±0.596	0.465±0.437
Soil (n=32)	-9.453±4.564	9.220±2.109

[a] Values shown are mean ± 1 s[5]

[b] Grass sample from “kinetic” experiment harvested after 14 days exposure

[c] Grass sample from “seasonal” experiment covering period specified

Figure 1: Plot of the Modulus of %ee in Grass versus Exposure Time in "Kinetic" Experiment for: (a) PCB# 95



(b) PCB# 149

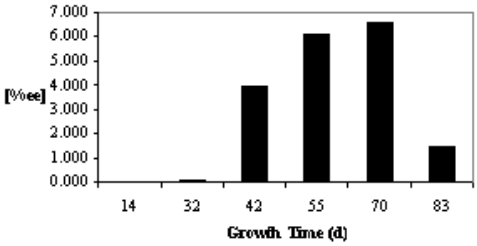
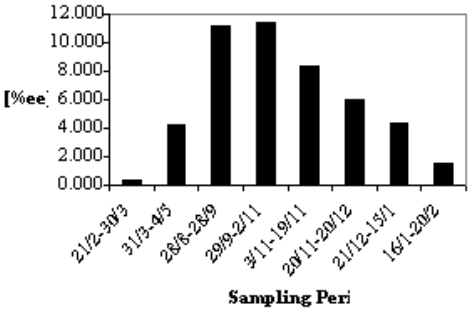


Figure 2: Plot of the Modulus of %ee in Grass Samples taken during the "Seasonal" Experiment for: (a) PCB# 95



(b) PCB# 149