PCDD, PCDF, AND DL-PCB IN TERRESTRIAL ECOSYSTEMS: ARE THERE CORRELATIONS OF LEVELS OR PATTERNS IN SOIL AND ROE DEER LIVER?

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Introduction

Recently it became obvious that sheep livers in Germany are highly contaminated with PCDD/F and PCBs[1], exceeding frequently maximum limits set by the European Commission[2]. Since no correlation could be found between liver contamination and grassing areas or husbandry, it has been concluded that a specific metabolism of sheep causes this high enrichment of PCDD/F and PCB in liver. Meanwhile, several national and international monitoring programs reported comparable high contamination of liver from other game, as e.g. deer and boar[3]. The German Environmental Specimen Bank (ESB) with its collection of terrestrial specimens offers the possibility to detect sources of contaminants and correlations of contamination[4]. Therefore we analyzed soil samples from the campaigns 2002 and 2006 and livers of roe deer from best matching sampling years for PCDD/F and dl-PCB. This paper gives an evaluation of respective levels and patterns in the organic layer of soil and roe deer liver and includes the results of previous analyzed conifer shoots[5] as an indicator for air pollution.

Materials and methods

Samples. The study was conducted on livers of one year old roe deer (*Capreolus capreolus*) and soil sampled in two urban-industrialized areas (U1, U2) and in seven different rural locations (R1-R7) from the north to the south of Germany (fig.1) and archived by the German ESB. Livers were collected annually or biannually since 1980 and processed under well defined and reproducible conditions according to standard operating procedures[6]. About ten livers per site were pooled, grinded and stored as homogenized powder in sub-samples of approx. 10g wet weight. Soil sampling in the frame of the ESB started in 2002 with a four year sampling period. Organic layers (defined as humus layer including roots), topsoil (A-horizons), and subsoil (B-horizons) were collected and processed as described[7] and stored as homogenized material in sub-samples of approx. 100g wet weight. Sampling and processing of conifer shoots has been described earlier[5]. Long term archiving is performed at temperatures below -150°C in an inert atmosphere resulting from evaporating liquid nitrogen.

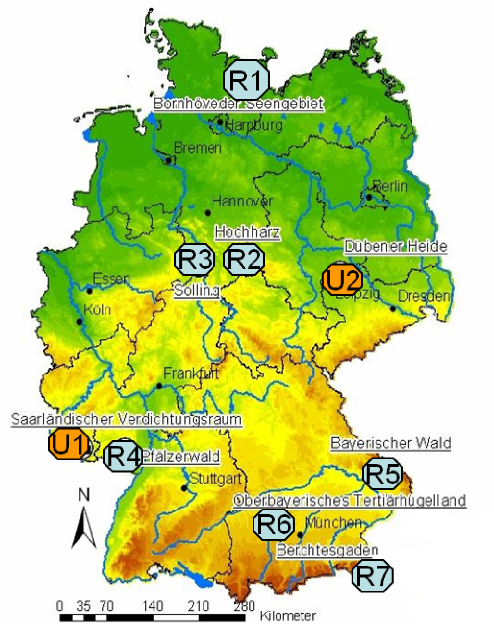

Figure 1 Sampling locations of soil, roe deer liver and conifer shoots. Urban areas: U1, Warndt/Saarland conurbation; U2 Dübener Heide Mitte. Rural areas: R1, Bornhöved; R2, Harz (~ 700 m above sea level); R3, Solling (~ 400 m a.s.l.); R4, Pfälzerwald (~ 270 m a.s.l.); R5, Bayerischer Wald (~1240 m a.s.l.); R6, Oberbayerisches Tertiärhügelland (~ 500 m a.s.l.); R7, Berchtesgaden (~1125 m a.s.l.).

Analysis. The analysis of the samples was performed by the Eurofins GfA GmbH dioxin competence center, Hamburg, Germany. Sample preparation and extraction was performed as follows: Soil samples were dried, ground and homogenized; deer liver was lyophilized prior to extraction. Extraction was performed via hot soxhlet extraction using toluene. The sample clean-up consisted of a multi-step column chromatography with column size and modification being adapted to the matrix (e.g. high capacity acidic silica column for fat removal from liver samples). Analysis was performed on Waters AutoSpec mass spectrometers at a mass resolution R ≥

10000 by isotope dilution quantification with every analyzed compound (exception: 1,2,3,7,8,9-HxCDD) having its own 13C-labelled internal quantification standard added to the sample before extraction. The deer liver method is according to EU food legislation. Overall analytical quality has been accompanied by a QA/QC-scheme with laboratory blanks as well as control analyses of reference materials.
Average differences between lower bound approach (values below limit of quantification (LOQ) were set 0) and upper bound approach (levels below LOQ were set as LOQ) were 0.6% (1.3% maximum) in organic layers of soil and 0.4% (maximum 4.2%) in roe deer liver. TEQs were calculated with TEFs from 1998 due to EU-Ordinance on maximum levels of food and with the upper bound approach. For comparison, levels in soil and deer liver were calculated with TEFs from 2005 too (Table 1).

Results and discussion

A summary of the WHO-PCDD/F-PCB-TEQ in organic layer and roe deer liver is provided in table 1. All 21 liver samples from 2 urban and 7 rural locations show a high contamination of PCDD/F and dl PCB and – taken as food - exceed the EU maximum levels set for liver of terrestrial animals[2]. The stacked data in fig.2 reveal that both, the respective limit value of 6 pg TEQ/g fat for PCDD/F as well as the limit of 12 pg TEQ/g fat for the sum of PCDD/F and dl PCB are exceeded. Dl-PCB-TEQs contribute between 25 and 48% (mean 40%) to sum TEQs, with PCB 126 accounting for about 95% of the PCB toxicity. PCDD/F-TEQs are dominated up to 95 % by furans, with the congener 2,3,4,7,8-PeCDF accounting for 60 to 80% of PCDD/F toxicity.
Organic layers of soil from 8 locations show a wide range of contamination (Fig 2). Surprisingly, neither the top layers from urban areas nor from high mountain locations but the samples from the low mountain range (R2-Harz, R3-Solling) offered the by far highest PCDD/F-PCB contamination. Dl-PCB-TEQs contribute between 14 and 35% (mean 23%) to sum TEQs, with PCB 126 accounting for 80 to 95% of the PCB toxicity. PCDD/F-TEQs are dominated by furans (share of 65 to 80%), with the congener 2,3,4,7,8-PeCDF accounting for 30 to 40% of PCDD/F toxicity.
In roe deer liver and organic layer from most locations we observed a tendency to lower WHO-TEQs in later compared to earlier 2000s years. However, the PCB- versus PCDD/F-TEQ ratios did not change with time and the patterns of single congener TEQs showed no major alterations. Especially in soil samples PCB- to PCDD/F-TEQ ratios as well as single congener TEQ patterns seemed very site-specific.

Table 1 Sum WHO-TEQ in roe deer liver and soil organic layer, calculated both with 1998s and 2005s TEFs

	Roe deer liver (n=21) pg TEQ/g fat				**Soil organic layer (n=16) ng TEQ/g dm**			
TEF 1998	**Mean (st.dev)**	**median**	**min**	**max**	**Mean (st.dev)**	**median**	**min**	**max**
PCDD/F	36.8 (19.4)	33.3	11.7	91.7	29.1 (27.2)	18.2	6.6	95.5
Dl PCB	24.4 (11.9)	23.8	7.9	53.0	7.1 (4.2)	5.6	1.9	18.4
Sum TEQ	61.2 (30.8)	54.1	19.6	144.7	36.1 (31.1)	24.8	8.5	114.9
TEF 2005								
PCDD/F	26.4 (13.3)	25.5	8.5	61.4	24.1 (22.4)	15.2	5.5	79.5
Dl PCB	23.9 (11.7)	23.2	7.7	52.0	6.7 (3.9)	5.4	1.8	17.1
Sum TEQ	50.3 (24.6)	45.6	16.2	113.4	30.9 (26.1)	21.4	7.4	96.6

PCDD/F- and dl-PCB-TEQs in deer liver and soil were not correlated (p-value > 0,1). High contaminated soil does not necessarily lead to a higher contamination of roe deer liver and vice versa. Since air is known as another possible source of PCDD/F and dl-PCB, we compared soil and liver results with respective TEQs in conifer shoots originating from the same sampling locations and investigated earlier[5] (fig.2). Statistical treatments revealed no correlations between conifer shoots and roe deer liver. Comparisons of soil and conifer shoots yielded significant correlations (p-value < 0.05) between dl-PCB-TEQs in the organic layer of soil and shoots.

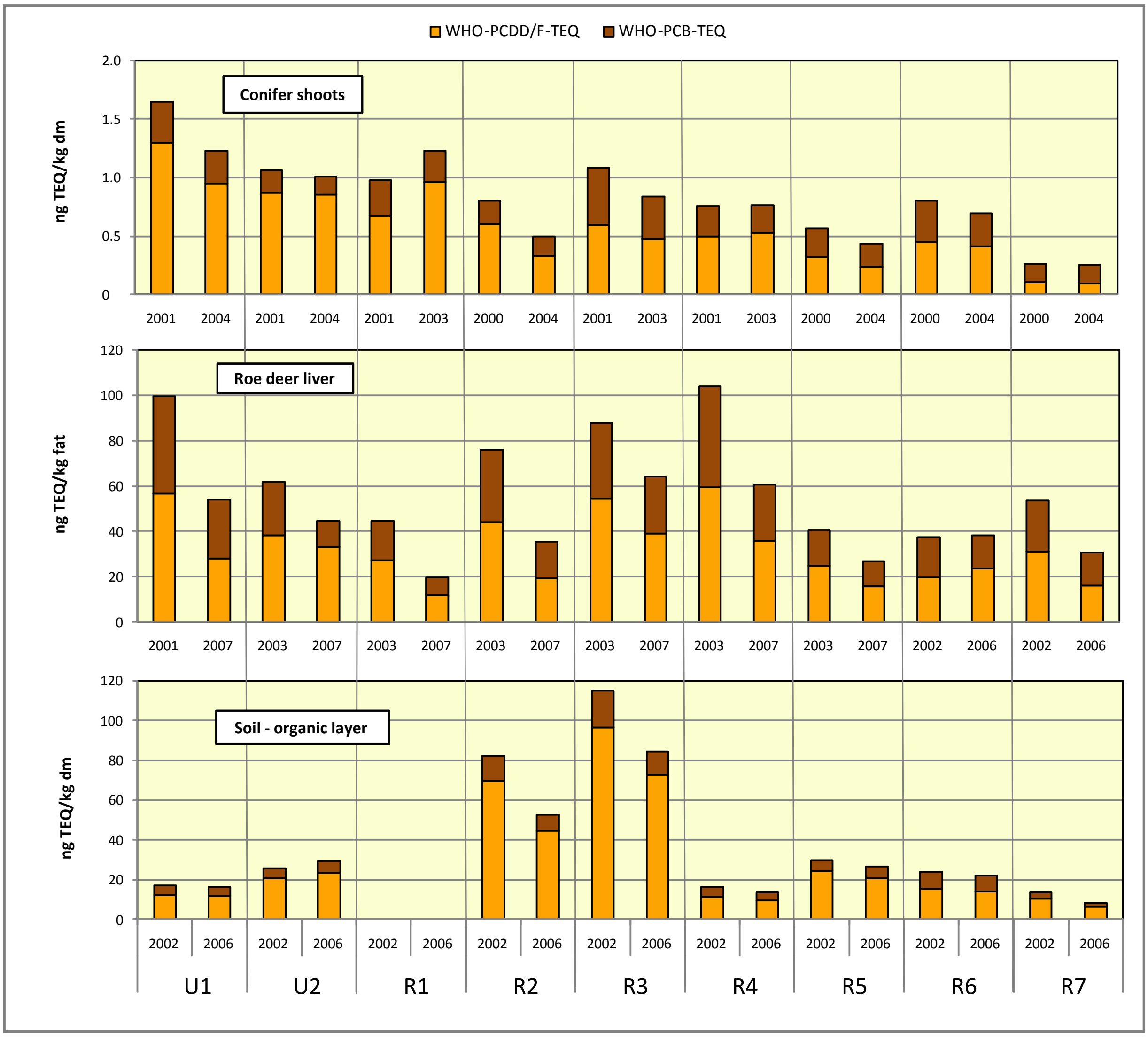

Figure 2 PCDD/F- and PCB-TEQ in conifer shoots, roe deer liver, and the organic layer of soil (organic layer not available from the agriculturally used area R1)

Furthermore we compared the concentrations and patterns of PCDD/F congeners in the three specimens. Figure 3 provides typical respective profiles in organic layer of soil, roe deer liver and conifer shoots taken from the location R6 (Oberbayerisches Tertiärhügelland) and the sampling year 2002. The congener patterns differ strongly in the three matrices. The congener profile from organic layer is typical for this matrix and in accordance with the average profile of the dioxin data base resulting from about 1.400 data sets (www.pop-dioxindb.de). In organic layers as well as in conifer shoots OCDD have the highest concentration, followed by HpCDD. In roe deer liver 2,3,4,7,8-PeCDF is the main congener followed by OCDD and HpCDD. This is different from the congener profile of sheep liver[1], which is more similar to soil maybe due to different eating behaviours.

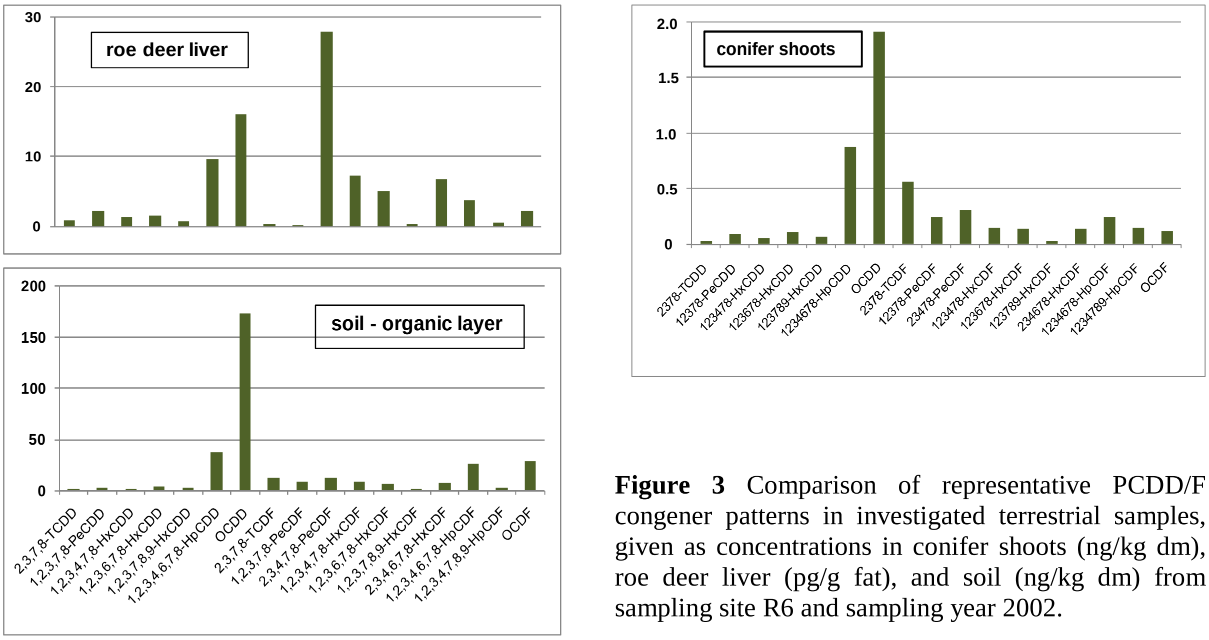

Figure 3 Comparison of representative PCDD/F congener patterns in investigated terrestrial samples, given as concentrations in conifer shoots (ng/kg dm), roe deer liver (pg/g fat), and soil (ng/kg dm) from sampling site R6 and sampling year 2002.

Conclusion

The liver of roe deer is highly contaminated with PCDD/F and dl-PCBs even in natural surroundings. The results show high loaded roe deers in low contaminated soil, and also in areas with high contaminated soil the liver is not higher burdened than in other regions. Looking for conifer shoots as an indicator for air contamination and first entry into plants there is also no correlation. This finding is very surprising and the cause cannot be explained but by a specific metabolism in liver. For sheep one source of PCDD/F and dl-PCB is the uptake of soil while they are grazing but this builds only a fraction of the contamination. Other sources are feed and still unknown causes. Roe deer have a different eating behavior. Their diet varies and includes less grass but more buds and leaves. The kids take milk from their mother for about 6 month or more but eat fresh leaves and herbs from the 2nd week of their life. High contamination of liver was the starting point to analyze roe deer livers. The study shows that respective high burdens are not only observed in sheep but also in other terrestrial mammalians. Furthermore, the fact that ESB sampling sites cover regions all over Germany indicate that there are no regional influences.

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