Bull. Environ. Contam. Toxicol. (1999) 63: 15-24

Environmental Contamination and Toxicology

PCDDs/PCDFs, Coplanar PCBs and PCBs in Barn Owl Eggs from Different Areas in the State of Brandenburg, Germany

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Received: 28 January 1999/Accepted: 4 May 1999

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are members of the same class of organochlorine compounds, the polycyclic halogenated aromatic hydrocarbons (PHAHs) of which tetrachlorodibenzo-p-dioxin (TCDD) is so far known the most toxic with regard to all vertebrates.

There are many known sources of dioxins and furans including for example combustion, high-temperature industrial processes or industrial chemicals like pentachlorophenol and polychlorinated biphenyls. Although polychlorinated biphenyls (PCBs) are no longer manufactured in most western states they remain ubiquitously distributed in the environment as well as the dioxins and furans.

Through atmospheric deposition and river transport these substances have spread throughout terrestrial and aquatic environments resulting in contamination of biota. Instead of relating any biological effect to the concentration of total PCBs in biota the contribution of constituting non-ortho and mono-ortho PCBs being equivalent to the action of TCDD is preferentially determined. Due to their physicochemical properties they bioconcentrate and bioaccumulate leading as well to biomagnification through trophic transfer. All toxicologically relevant PHAHs are for example transported across the barrier of blood-placenta or blood-mammary gland or are transferred into the yolk of bird eggs. Numerous data on the concentrations of these substances in eggs of eagles, cormorants or herons have been reported (Bosveld and van den Berg, 1994; Hoffman *et al.*, 1996; Elliot *et al.*, 1998 and 1996; Elliot *et al.*, 1997 and Custer *et al.*, 1997). However, respective data from eggs of the barn owl (Tyto alba) as a perennial and predatory species occupying agricultural sites are so far unknown.

In the present investigation eggs were collected from comparable habitats of this bird. The respective breeding populations have been monitored for several years and the prey of barn owls has been characterized in such a way that conclusions on the kind and extent of transfer of the PHAHs through the food web could be drawn.

Because the barn owls were nesting within comparable small-scale mixed farmlands being undisturbed by conversions it is of interest if their eggs could be used as indicators of local contamination.

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MATERIALS AND METHODS

The habitats of barn owls were characterized mainly by agricultural landscapes. Eggs were collected in 1995 and 1996 from two areas situated in the eastern and in the western part within the state of Brandenburg. Steinhöfel (UM), Günterberg (GÜ), Bruchhagen (BR) and Herzsprung (H) are within a circle of 15 km and represent the eastern area, Buckow (BU) on the other hand is the only place in the western area.

The total content of the eggs were freeze-dried and soxhlet-extracted with toluene/isopropanol (80/20 v/v) after ^{13}C-labelled PCDDs/PCDFs and PCBs had been added as quantitative standards. The resulting toluene/isopropanol extract was evaporated. For gravimetric fat determination the diethylether soluble part of the extract was used. The residue was taken up in hexane and then refluxed on silica/sulphuric acid (44%), followed by chromatography on a carbon column (forward elution with hexane/ethylacetate for removing lipids, also containing PCB-fraction I, backward elution with toluene yielding the PCDDs/PCDFs and the coplanar PCBs). This was followed by chromatography on 5 g Alumina B Super I for Dioxin Analysis (elution with 40 ml pentane, 75 ml hexane / dichloromethane 98/2 v/v, containing the PCB-fraction II and with 50 ml hexane / dichloromethane 50/50 v/v containing the PCDD/PCDF and the coplanar PCB-fraction). PCB-fraction I and II were combined and the solvent was completely evaporated. Another clean up step was performed by chromatography on 25 g Alumina B Super I for Dioxin Analysis (elution with 100 ml hexane and 300 ml hexane / dichloromethane 98/2 v/v containing the PCBs). The PCDD/PCDF-results are given in international toxicity equivalents (I-TCDD-EQs or I-TEQ, *NATO CCMS*, 1988), the amounts of the coplanar PCB congeners No 77, 126 and 169 as TCDD-EQs (TE or TEQ) according the proposal of a WHO-ECEH working group (Ahlborg *et al.*, 1994). The results of the PCB-congeners PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180 are given in µg/kg. Statistical analysis (principal component analysis, box and whisker plots) was performed using SPSS (SPSS Inc., Chicago, IL, U.S.A.)

RESULTS AND DISCUSSION

In Table 1 the concentration of the 17 analyzed 2,3,7,8-chlorinated PCDDs/ PCDFs, the 3 coplanar PCBs and the 6 nonplanar PCBs as measured in the sampled eggs are shown. The numbers assigned to the PCDD and PCDF congeners follow the proposal of Bather and Ballschmiter (1992).

The concentrations of the various 2,3,7,8-chlorine substituted congeners of PCDDs and PCDFs in each of the analyzed eggs contribute in a comparable manner to the TCDD toxicity equivalents (TCDD-EQs) irrespective of the sampling location. Major PCDD contaminants of toxicological relevance were found to be 1,2,3,7,8-PnCDD and 2,3,7,8-TCDD. 2,3,4,7,8-PnCDF is the major dibenzofuran whereas the PCB congener 126, 3,3‘,4,4‘-PnCB, is the mostly

Table 1. Analytical results

Egg description	Gü	UM	BU1	BU2	H1	H2	BR1	BR2
Weight (g)	**17.59**	**14.26**	**19.13**	**17.79**	**18.13**	**17.86**	**14.15**	**16.19**
Volume (cm³)	**20.13**	**20.13**	**21.45**	**20.91**	**20.37**	**20.91**	**18.38**	**20.13**
Fat content (%)	**6.84**	**7.45**	**5.56**	**4.47**	**4.19**	**7.58**	**6.51**	**3.79**
wet weight (g)	**13.9**	**11.0**	**16.9**	**13.7**	**14.1**	**15.2**	**11.7**	**12.4**
dry weight (g)	**2.88**	**2.39**	**3.15**	**2.68**	**2.2**	**3.35**	**2.79**	**2.24**
PCDDs and PCDFs and TEs (unit: pg/g wet weight)								
Dioxin # 48	0.78	0.40	0.56	0.27	0.46	0.36	0.43	0.26
Dioxin # 54	1.4	1.0	1.7	0.60	1.3	0.94	1.2	0.56
Dioxin # 66	0.72	1.1	1.6	0.57	1.2	0.89	1.2	0.54
Dioxin # 67	0.86	1.0	2.5	0.49	1.1	0.91	1.4	0.65
Dioxin # 70	0.10	0.06	0.23	0.07	0.12	0.10	0.16	0.09
Dioxin # 73	0.83	0.50	2.4	0.51	1.3	2.1	5.2	3.0
Dioxin # 75	1.8	1.3	2.1	1.2	8.6	12	29	16
Furan # 83	0.70	0.84	2.1	0.80	1.1	0.85	1.2	0.48
Furan # 94	0.18	0.18	0.58	0.23	0.25	0.23	0.26	0.12
Furan # 114	6.9	17	11	6.3	11	10.0	16	6.2
Furan # 118	0.90	1.4	1.1	0.64	1.5	1.3	1.7	0.58
Furan # 121	0.73	1.4	1.1	0.66	1.4	1.3	1.8	0.61
Furan # 130	0.69	0.64	0.62	0.38	0.76	0.65	0.86	0.37
Furan # 124	0.02	0.04	0.05	0.01	0.01	0.02	0.02	0.01
Furan # 131	0.26	0.13	0.18	0.14	0.20	0.22	1.4	0.33
Furan # 134	0.03	0.03	0.13	0.04	0.06	0.05	0.07	0.04
Furan # 135	0.13	0.13	0.41	0.14	0.13	0.13	0.38	0.20
I-TEQ	**5.4**	**10**	**7.8**	**4.1**	**7.6**	**6.5**	**10**	**4.0**
TE (copl. PCB)	**6.6**	**15**	**18**	**6.1**	**8.2**	**6.7**	**14**	**5.9**
PCBs: (unit: ng/g wet weight)								
PCB 77	0.037	0.015	0.130	0.012	0.068	0.030	0.046	0.032
PCB 126	0.064	0.142	0.170	0.059	0.079	0.065	0.130	0.057
PCB 169	0.015	0.035	0.056	0.025	0.022	0.014	0.048	0.019
PCB 28	0.49	0.40	0.47	0.30	0.43	0.52	0.53	0.22
PCB 52	0.27	0.32	0.14	0.23	0.27	0.20	0.35	0.11
PCB 101	1.1	1.2	0.79	0.80	0.66	0.61	1.3	0.57
PCB 138	17	36	41	25	13	14	39	20
PCB 153	28	87	121	65	29	28	100	41
PCB 180	12	34	62	41	17	17	57	23

concentrated congener among the coplanar PCBs.

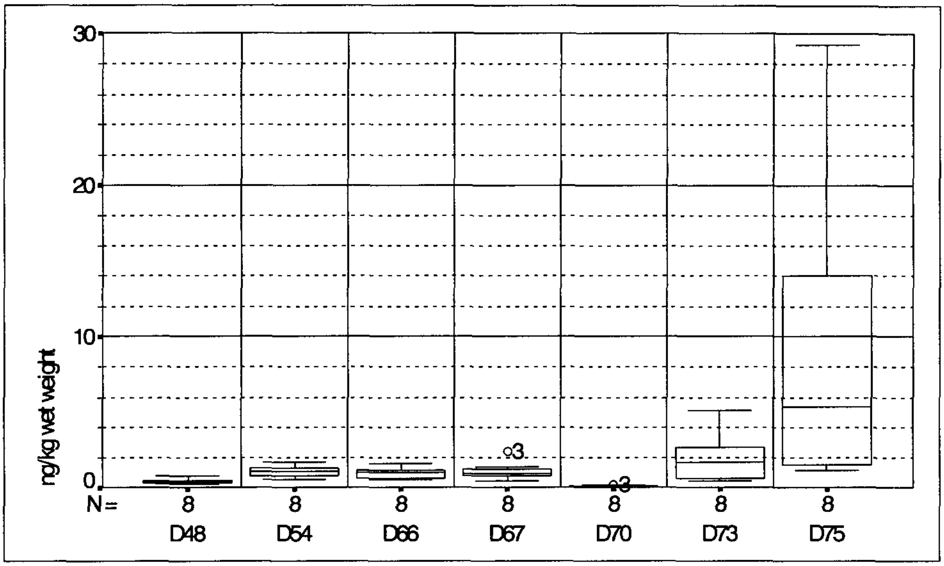

Figure 1. Box Whisker Plot of the PCDD congener profile

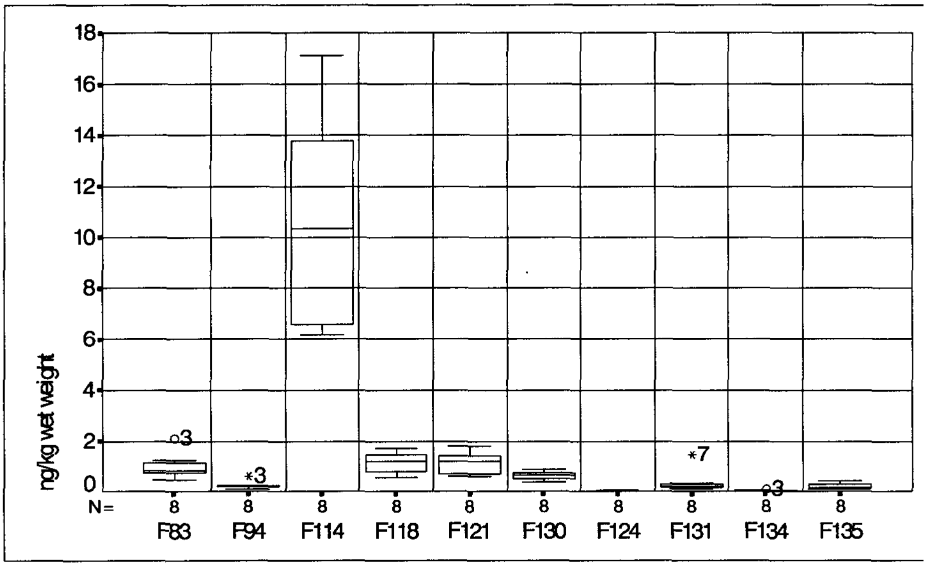

Figure 2. Box Whisker plot of the PCDF congener profile

The Box and Whisker plots in Figure 1 and 2 show the congener profile of PCDDs and PCDFs, respectively. The concentrations of PCDDs are rather low if compared to those in eggs of peregrine falcon as an other top predator (Jarman *et al.*, 1993). However, the concentrations of higher chlorinated dibenzofurans in

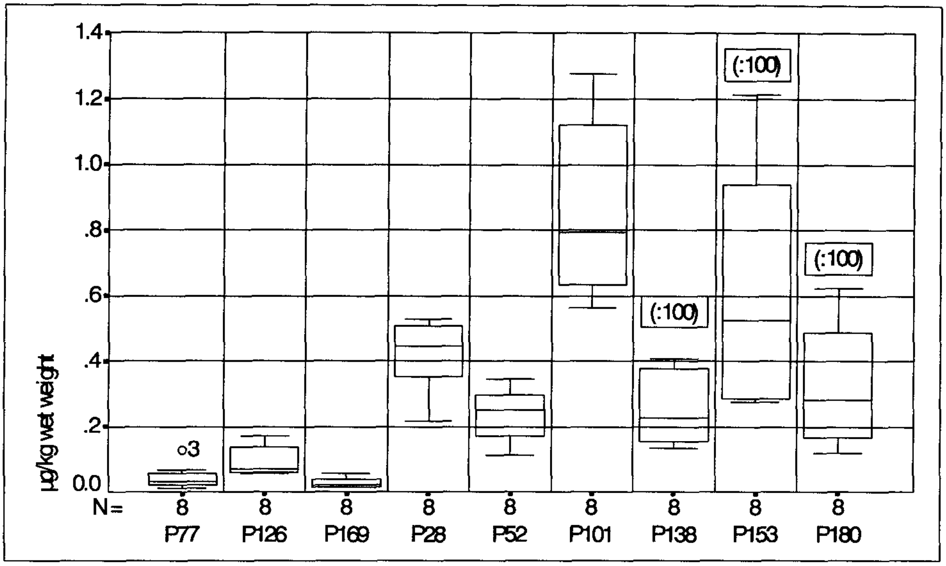

Figure 3. Box Whisker Plot of the PCB congener profile

both species are mostly comparable but lower than that of PCDDs. However, in both species there is a great elevation of the amounts of 2,3,4,7,8-PnCDF which was also observed in eggs from some fish-eating birds like bald eagle (Haliaetus leucocephalus) (Elliot *et al.,* 1996), double-crested cormorants (Phalacrocorax auritus) (Yamashita *et al.,* 1993; Sanderson *et al.,* 1994) or common terns (Sterna hirundo) (Bosveld *et al.,* 1995).

The congener profile of 6 nonplanar and 3 coplanar PCBs is shown in Figure 3. The height of plots belonging to congeners 138, 153 and 180 are reduced by a factor of 100 for better adaption into the figure. Among the coplanar congeners 3,3‘,4,4‘,5-PnCB (No 126) is obviously dominating. Ratios of PCB 126/PCB 77 or PCB 126/PCB 169 in eggs from barn owls are much higher than one. Similar results were received for eggs of double-crested cormorants nesting in regions which are assumed to be contaminated by PHAHs (Williams *et al.,* 1995a) as well as in the eggs of bald eagle (Elliot *et al.,* 1996). Though the congener 77 is reported to be easily metabolized with an efficient rate of clearance (Boon *et al.,* 1997) there exist conflicting reports, showing that in eggs of red-breasted mergansers (Mergus serrator) (Williams *et al.,* 1995b), of Caspian terns (Hydroprogne caspia) (Yamashita *et al.,* 1993), of black guillemots (Cepphus grylle L.) (Koistinen *et al.,* 1995) the ratio of PCB 126/PCB 77 is much smaller than one. In eggs of common terns and of Forster’s tern (Sterna forsteri) collected from similarly contaminated sites at Lake Michigan, inverse relationships regarding this ratio were observed (Ankley *et al.,* 1993; Tillit *et al.,* 1993). Bioconcentration factors developed for spottail shiner (Notropis hudsonius) to Forster’s tern eggs reveiled the following ratios: congener 77 = 0.17; congener 126 = 64; congener 169 = 176. The bioconcentration factor for 2,3,7,8-TCDF was

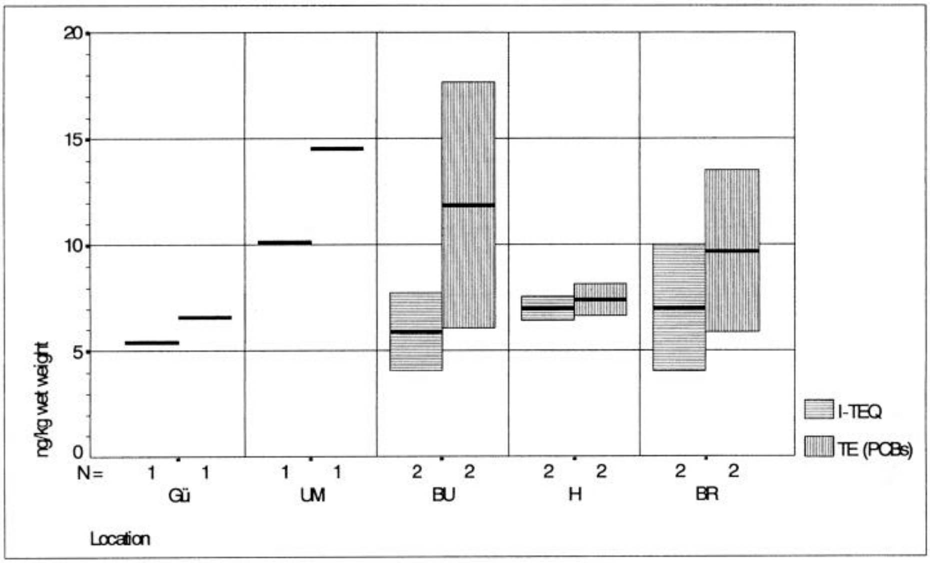

Figure 4. Results in order of the sampling location

clearly negligible (Kubiak *et al.,* 1989). The diminished clearance of congener 77 as mentioned above may either be caused by a modified xenobiotic metabolism due to the presence of further substances in the mixture of contaminants or may be an inherent quality of these species. However, from the present data it is evident that the congener 126 is enriched by the barn owl to a greater extent than even the recalcitrant congener 153. In eggs of those species with high ratios PCB 126/PCB 77 the ratio of PCB 126/PCB 153 appears always to be higher than $1x10^{-3}$ Selective retention of coplanar congener 126 is consistent with laboratory data for rats where fifteen times greater hepatic retention of the coplanar congener 169 was observed relative to congener 153 (DeJong *et al.,* 1993).

The analyzed barn owl pellets from the respective nests indicated that the main prey species at all locations were the vole rat (Arvicola terrestris) and the common vole (Microtus arvalis). Remnants of the common shrew (Sorex araneus) were found to account between 6 and 30%. Therefore the barn owls were feeding mainly on small herbivorous mammals resulting in a much lesser burden by high lipophilic compounds like PCBs and PCDDs/PCDFs. The dominant prey species themselves may be burdened essentially by contaminated soil. The exposure of barn owls via this short pathway within the food chain therefore appears useful in deriving soil quality criteria from the chemical analysis of their eggs.

In Figure 4 the TCDD-EQs contributed by PCDDs/PCDFs as well as by the dioxinlike coplanar PCB-congeners 77, 126 and 169 are shown. At all locations these PCB congeners accounted for more than 50% of the calculated TCDD-EQs in the barn owl eggs. In studies which have quantified all Ah-receptor-active PCB-congeners it has been found that these congeners have accounted for 70-95%

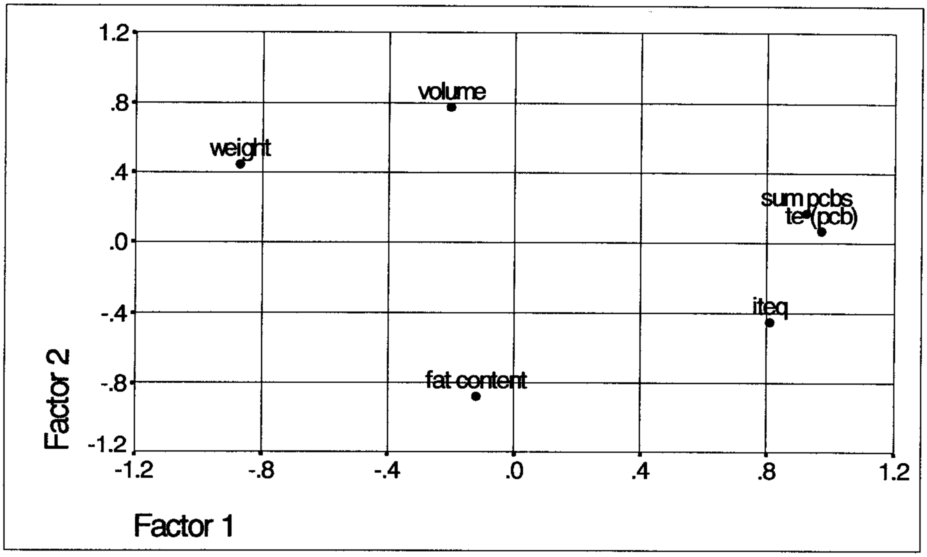

Figure 5. PCA-analysis of the components weight and volume of the eggs, fat content, and contamination

of the calculated TCDD-EQs, based on the additive model while the I-TCDD-EQs contributed by PCDDs/PCDFs accounted for the rest of the TCDD-EQs (Smith *et al.,* 1990). Studies comparing the predicted TCDD-EQs with those derived from bioassays yielded a fairly good agreement (Brunström *et al.,* 1992).

The interdependency of the various parameters measured in the sampled eggs of barn owls is best described by principal component factor analysis. In this case we are dealing with a six variable problem: egg weight, egg volume (calculated from the diameters), lipid content, I-TCDD-EQs, TCDD-EQs (PCB) and the sum of PCBs including 3 coplanar and 6 nonplanar congeners. Because a two factor solution accounts for 84% of the variance a graphical portrayal of two independent factors will most clearly describe the interdependency of the parameters.

The high factor loadings of I-TCDD-EQs, TCDD-EQs (PCB) and the sum of PCBs represent a very strong correlation among these parameters. The negative loading of weight or volume means that the group of different congeners is negatively correlated. A similar relationship between egg volume and total TCDD-EQs in egg yolk has been described for populations of common terns from Netherland (Bosveld *et al.,* 1995). Total concentrations of PCBs may not provide the best correlation with the degree of reproductive impairment because only the coplanar PCBs and other toxic PHAHs have been directly linked to these anomalies (Hofmann *et al.,* 1996). The results given in the present report, however agree well with other investigations in which a statistically significant correlation was observed between concentrations of total PCBs and concentrations of three coplanar PCBs with the greatest potency to cause toxicity in mammals (Tanabe *et al.*, 1987).

Acknowledgments. The expert technical assistance by M. Schulz and M. Winkler is gratefully acknowledged.

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