Bull. Environ. Contam. Toxicol. (1997) 58:219-226

Environmental Contamination and Toxicology

Organochlorines in Eggs and Food Organisms of Avocets *(Recurvirostra avosetta)*

S. Dietrich,[1] A. Büthe,[2] E. Denker,[2] H. Hötker[3]

[1]FB Biologie, Philipps Universität Marburg, K. v. Frisch Strasse, D-35043 Marburg, Germany
[2]Chemisches Institut, TH Hannover, Bischofsholer Damm 15, D-30173 Hannover, Germany
[3]Forschungs- u. Technologiezentrum, Universität Kiel, Hafentörn, D-25761 Büsum, Germany

Received: 30 June 1996/Accepted: 20 September 1996

Compared to other wader species the eggs of Avocets *(Recurvirostra avosetta)* differ both in composition and concentration of their PCB-load (Becker et al. 1991, Focardi et al. 1988). One explanation might be the contamination at the wintering sites (Denker et al. 1994).

Studies on more than 400 individually colour ringed Avocets allowed following many birds both at their breeding site in the German Wadden Sea and their main wintering sites along the French and Portuguese Atlantic Coast.

The approach was to analyse the eggs of those females whose past wintering site was known and to take samples of their typical diet at each of the study sites. Two questions were addressed: Is there a difference in the composition and amount of PCBs in the diet at the various sites? Is there a relation between the diet and the pollutants in the eggs?

Few data of contamination of Avocets are available and none refer to individual birds. Our results indicate that the differences from other wader species are not caused by the wintering sites only. The choice of their feeding habitat and their specialisation and preference for very soft substrate combined with the accumulation of PCBs in fat tissues of their dominant prey provide several 'PCB-traps' along their flyway. This paper shows a distinct variation in the PCB-pattern in different European coastal areas. There is no uniform distribution of these pollutants.

MATERIALS AND METHODS

The wintering sites sampled in this study are the Estuaries of the rivers Seine, Vilaine, Loire and the Baie de L'Aiguillon in France, and the Estuaries of the rivers Tejo and Sado in Portugal. Female Avocets usually spend about five months at their wintering site and return to their nesting site in March or April in order to start breeding four weeks later. During breeding seasons 1991 and 1992, twenty eggs of fourteen individuals were collected at the breeding site near Husum, German Wadden Sea (54° 33 'N, 08°55'E). The samples were coded by a capital letter for each individual bird and a running number for each egg. One egg per clutch was taken at random, except one complete 3-egg clutch (K 14-K16) swept off the nest

Correspondence to: S. Dietrich

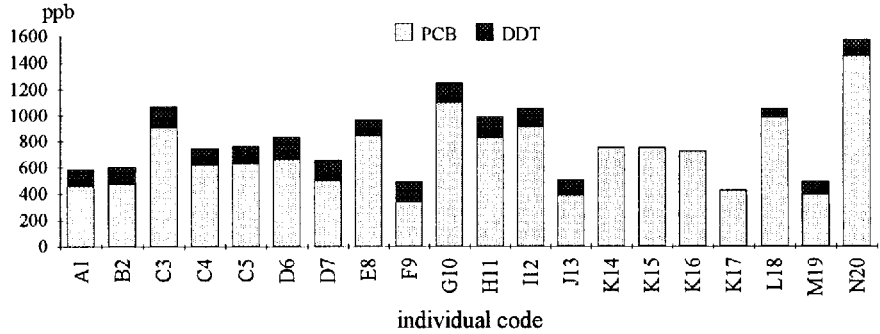

Figure 1. ∑ PCB and ∑ DDT in Avocet eggs of first and repeat clutches (n=20)

by a high tide was taken, too. Twice, an egg of each the first and the repeat clutch (C3/C4,K15/Kl7) of the same female could be sampled in the same year and twice eggs of the same individuals were collected in both breeding seasons (C3/C5, D6/D7). If not mentioned specifically, only eggs of the first clutches (n=16) were used for mean values and statistics. Forty-six pooled samples of typical Avocet food organisms (Polychaetes, Crustaceans, Molluscs, Chironomid larvae) were taken from different areas where flocks of Avocets and especially the birds of this study definitely had been observed feeding. It should be noted that only organisms of suitable size for Avocets were taken for analysis. Therefore the results do not represent the contamination of the benthos at the feeding sites in general. All samples were frozen at - 18°C and analysed according to the method described by Büthe & Denker (1995). The preparation of the egg samples was carried out according to Heidmann (1986), the food samples were prepared according to Denker (1996). In total 115 single congeners or groups of congeners where no further separation was possible were checked. Besides ∑ DDT, ∑ HCH, HCB and OCS were analysed as well. The results for the eggs refer to fresh weight without shell, for the food organisms to lipid tissue, all given in ppb (µg/kg).

RESULTS AND DISCUSSION

Avocet eggs of our study site showed relatively low levels of organochlorine contamination. However, they were still contaminated enough to be excluded as a potential human food according to German food control legislation (BGA 1988).

Table 1. Organochlorines except PCBs in Avocet eggs and food samples of different study sites (in ppb)

	food samples from			eggs
	France	Portugal	breeding site	
	(n=10)	(n=11)	(n=12)	(n=16)
αHCH	34.6	81.7	—	—
βHCH	275.4	227.3	13.2	0.7
γHCH	—	—	95.2	9.0
OCS	—	—	—	0.9
HCB	376.3	123.5	22.7	7.5
∑ DDT	1523.0	1102.3	190.1	126.8

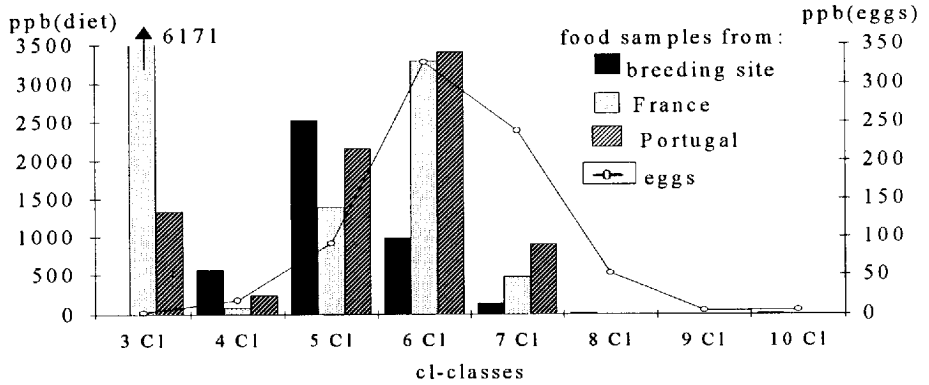

Figure 2. PCB concentration in Avocet eggs and food samples of different study sites according to chlorine classes

The arithmetic mean level of Σ PCB was 725 ppb, ± 304 ppb including all eggs (n=20). For first clutches only it was 748 ppb, ± 319 ppb (n=16). There was no significant difference between the eggs collected in 1991 and 1992 (t-test, $p > 0.2$). Other organochlorines were not detected at all or occurred on a low level (Table l). If detectable they decreased from the first to the repeat clutches.

Only two birds (M, N) spent most of the winter in France, with the last sighting at the end of January. They might have escaped from frosty days to Portugal for the rest of the winter. The values of Σ PCB in the eggs of these two birds represent a minimum and the maximum of all samples (Fig, 1). It was not possible to prove any differences among the wintering sites.

When the congeners are divided into groups according to the degree of chlorination hexa- and heptabiphenyls were apparently dominant in Avocet eggs (Fig. 2). The mean degree of chlorination in the eggs was 6.3. Low chlorinated biphenyls were hardly found. A peculiarity in Avocet eggs was the high amount of octabiphenyls. Comprising 7% of the Σ PCB it was 5-7 times higher than in other wader eggs (Becker 1991). Congeners with 9 and 10 chlorine atoms constituted about 1.5% of Σ PCB in the eggs. They have not been analysed in former studies; therefore, no comparison is possible. In general a comparison to former studies is difficult due to the lower number of congeners that were checked previously. A reduction to six indicator congeners only (pcb 28,52,101,138,153,180) that are

Table 2. Quantitative and qualitative comparison of PCBs in Avocet eggs and food samples of different study sites

	food samples from			eggs
	France	Portugal	breeding site	
	(n=10)	(n=11)	(n=12)	(n=16)
Σ PCB	11408 ppb	8025 ppb	4238 ppb	748 ppb
∅ cl-degree	5.0	5.2	6.0	6.1
# congeners	26	64	49	68
% dominant PCBs*	19%	35%	17%	60%

*PCB 118, 132/146, 138, 153, 160/163/164, 175/187, further explanation see text

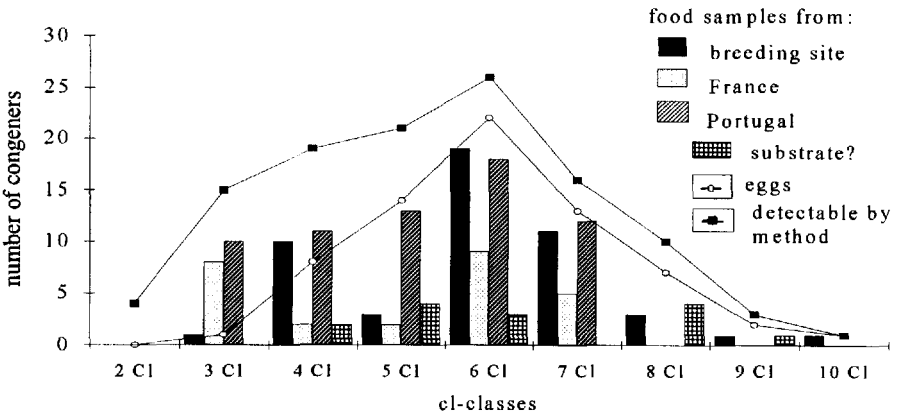

Figure 3. Number of congeners detectable by the employed method, found in Avocet eggs and in the food samples of different study sites, according to cl-classes. 'Substrate?' indicates those congeners which were found in the eggs but not in the food samples.

normally chosen for food control is not sufficient because these congeners constituted only 21% of Σ PCB in Avocet eggs. Changes are hardly detectable by this method.

Twenty-seven of 115 single congeners with a mean cl-degree of 4.7 were identified neither in the food samples nor in the eggs. Nineteen congeners (Ø cl-degree 3.9) were only detected in the diet but not in the eggs. Most of them belong to the low chlorinated biphenyls which are soon metabolized or eliminated by the birds' metabolism (Subramanian 1987). The food samples show significant differences between the areas in Σ PCB ($p=0.049$), in the degree of chlorination ($p=0.001$) and number of congeners ($p=0.025$). The quantitative contamination was highest in France, followed by Portugal and the breeding site (Table 2). Half of the Σ PCB concentration in the French samples originated from tribiphenyls. Even in the eggs of the two birds wintering in France this chlorine class was only represented by PCB 28 with less than 0.1%, which supports the fact of rapid metabolization or excretion of low chlorinated biphenyls in birds. The qualitative aspects of the PCB pattern reflects a shift from low chlorinated PCBs in the French samples towards an increasing degree of chlorination in food samples of Portugal and the breeding site. The highest proportion of the congeners detectable by our method were found in Portuguese food samples and in the eggs (Fig. 3). Fifty-four congeners (Ø cl-degree 6.0) were quantified both in the eggs and the diet. Ten of them include

Table 3. Contamination of Avocet eggs according to the origin of the congeners

	all sites	Portugal & breeding site	Portugal & France	Portugal	breeding site	not detected in the diet
Σ congeners	12	13	2	8	8	15
Ø cl-degree	5.8	5.8	5.5	5.3	7.6	6.2
% of Σ PCB in the eggs	47.6	34.5	1.5	9.2	2.0	5.2

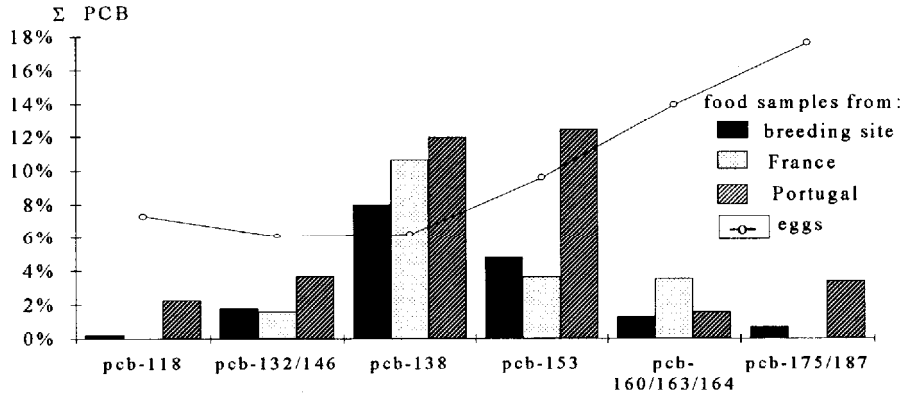

Figure 4. Dominant PCBs in Avocet eggs and food samples of different study sites

more than 60% of Σ PCB in the eggs with a cl-degree of 6.1. In the following they are called 'dominant PCBs' (Fig. 4). In the food samples they were less dominant but present at all sites.

It is possible to classify six groups of congeners within the benthos samples according to their origin and to compare these groups with the PCB contamination of the eggs (Table 3). Twelve congeners which represented 47.6% of Σ PCB in the eggs were found at all study sites, followed by 13 congeners found in the diet of Portugal and the breeding site. Both groups covered about 82% of ∑ PCB in the eggs with a mean cl-degree of 5.8. The contamination could have happened at any time. Therefore it is not possible to determine the origin of most of the PCB load in the eggs. The uptake rate and turnover rate of the congeners is not known. The 'available PCBs' were highest in the Portuguese diet but if these PCBs had been metabolized within a few weeks the contamination of the eggs should refer to the prey of the breeding site. In spite of a possibly rapid metabolization there was a permanent input which explains the high amount of hexa- and heptabiphenyls in Avocet eggs.

There was no congener specific for France. Only two congeners were quantified both in France and Portugal. Together with eight congeners found only in Portuguese food samples they were a potential burden exclusively brought from the wintering sites; 10.7% of Σ PCB in the eggs belong to these congeners.

Congeners specific for the breeding site were characterized by a high content of cl-atoms (Ø cl-degree 7.2). Octa- to decabiphenyls were found only in food samples of the breeding site which had been taken in a reservoir close to the nesting areas. Chironomid larvae were the most contaminated species. This pond was frequently used by the birds as a feeding area. During the time of research there was intensive construction work by hydraulic machines which were a potential source of PCBs. Additionally, ditches from one of the most intensive agricultural areas in this region were drained into this reservoir.

The last category of 15 chlorinated biphenyls (Ø cl-degree 6.2) was not detected in the food samples at all but covered 5.2% of ∑ PCB in the eggs. They may have been under the limit of determination in the diet but could be quantified in the eggs due to biomagnification. Another possibility of their occurrence in the eggs might

be the input directly with the substrate (see Fig. 3) due to the aggregation of PCBs at fine particles (Duinker 1986). Avocets do not forage very selectively but also swallow mud and non-digestable particles like threads and plant material in a notable amount (Moreira 1995).

The possibility of the contamination with highly chlorinated biphenyls at the breeding site is supported by the comparison of the first and second clutches of two females. Although only two repeat clutches were examined the data of these individuals give a hint to the natural loss rate of PCBs under field conditions. The Σ PCB decreased from the first to the second clutch about 32% (C4) and 44% (Kl7). Apparently, laying eggs is a suitable way for birds to rid themselves of xenobiotics. On the level of cl-classes, congeners lower than pentabiphenyls were reduced more than 50%, hexabiphenyls about 45% and heptabiphenyls about 23% which agrees well with the general mode of metabolism (DFG 1988). The highest chlorinated congeners were different in the two birds: Female 'C' was breeding in close neighborhood of the above mentioned reservoir and had a longer residence time of about 30 days until starting the second clutch. There was an increase of 9% (7.1 ppb) in the congeners specific for the breeding site. Congeners typical for the wintering sites were reduced about 50% (30.5 ppb). Female 'K' was breeding some km farther south, probably feeding in the Wadden Sea and laying the second clutch just one week after the loss of the first one. In this bird the congeners typical for the breeding site diminished about 13% (5.6 ppb). It should be considered that the absolute value is very low and the variation of these congeners is 4.2 ppb within the three eggs of the complete clutch. Congeners of the wintering sites reduced about 56% (45.1 ppb).

Figure 4 illustrates in more detail the concentration of ten dominant congeners in the eggs and the food samples.

Due to their chemical structure the congeners are metabolized in a different intensity (Safe & Hutzinger 1987). Comprising 17% of the Σ PCB in the eggs, heptabiphenyls 175/187 are the most important group of congeners within the dominant PCBs. However, they occurred in low concentration in the diet. This strong bioaccumulation in the eggs is possibly caused by the more complicated way of metabolism of these congeners.

In general the contamination at the breeding site was less than in Portugal. The comparison of the first and the repeat clutches indicates the influence of the local PCB contamination at the nesting site. All dominant congeners were reduced in the second clutches. PCB 118, one of the coplanar congeners which is relatively difficult to metabolize was found at low concentration in the Portuguese diet and nearly absent at the breeding site. It accumulated in the eggs to 7% of Σ PCB. There was a reduction of about 60% from the first to the second clutches which indicates a slow metabolic rate because the input at the breeding site should have been extremely low so most of the contamination originated of the wintering site. The reduction was equal to both individuals independently of their different residence time at the breeding site. There might be no other way to excrete this congener in considerable concentration than laying eggs.

PCB 138 was quantified in all food samples on a high level of 8%-12% of ∑ PCB. In the eggs it is represented with a mean value of only 6% of ∑ PCB. In spite of the permanent input there was a decrease of 30% from the first to the second clutches which was equal in both individuals. PCB 153 which is more difficult to

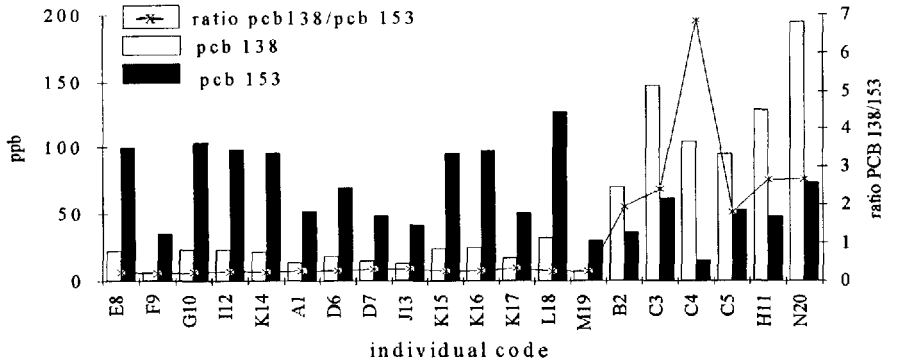

Figure 5. Ratio of PCB138/PCB 153 in Avocet eggs

metabolize than PCB 138 (no vicinal 'free' position) accumulated in the eggs to 10% of ∑ PCB. The contamination via the food at the wintering site was similar to PCB 138. The breeding site was less contaminated. The decrease to the second clutch was different in the two females: Bird "C" with a 4-week interval between the two clutches reduced PCB 153 about 75%, bird "K" which started breeding again after one week reduced it about 46%. It is also possible that inter-individual variability in metabolization was of greater importance than the residence time. An aspect of inter-individual differences occurs in the ratio of the two most important congeners, too. In 14 eggs of ten individuals the ratio of PCB138/PCB 153 is 0.2-0.3 constantly. In six eggs of four individuals it changes to 1.9-2.6 with an extreme ratio of 6.9 in the second clutch of bird "C" (Fig.5).

The PCBs in the eggs of Avocets originated both from the wintering site and the breeding site. Most of the congeners were present everywhere. In this study the highest chlorinated PCB-mixture was found in a reservoir at the nesting site. The contamination was not only a question of the locality but of the birds specialisation for foraging in the surface of fine substrate. Avocets prefer to feed in shallow freshwater or brackish ponds, in the muddy parts of estuaries and in muddy bays. Abiotic factors like higher temperature and low salinity favour the solution of PCBs at those sites (Falkner & Simonis 1982, Pavlou& Dexter 1979). Moreover, these areas are characterized by an enormous impact of human activities. PCBs aggregating on fine particles of the mud (Duinker 1986) were swallowed directly by the Avocets while feeding or via the benthos organisms living in the upper layer of the substrate. PCBs also accumulate in the fat tissue and in gonads (Larsson et al. 1993). Before leaving the wintering sites Avocets feed on fertile Polychaetes and Crustaceans for 4-6 weeks. On arrival at the breeding site their main prey is fertile, too and rich in fat tissue. Generally the permanent input of fat and therefore highly contaminated diet combined with the specialized foraging behaviour and the impact of human activities at the main feeding sites leads to cumulative effects in PCB contamination in Avocet eggs.

Acknowledgments. This study was financially supported by the Stifterverband für die Deutsche Wissenschaft, Essen and the Forschungs- und Technologiezentrum, Universität Kiel.

REFERENCES

Becker, P. H., Koepf, C., Heidmann, W. A., Büthe, A. (1991) Schadstoffmonitoring mit Seevögeln. Texte 2/92 Umweltbundesamt Berlin.

BGA (1988) Verordnung über Höchstmengen an Schadstoffen in Lebensmitteln (Schadstoff-Höchstmengen-Verordnung-ShmV) BGBl I:422-424.

Büthe, A., Denker, E. (1995) Qualitative and quantitative determination of PCB congeners by using a HT-5 GC column and an efficient quadruple MS. Chemosphere, Vol.30, No.4: 753-771.

Denker, E. (1996) Untersuchungen zur PCB-Belastung verschiedener Trophiestufen in der Nordsee bei Helgoland unter besonderer Berücksichtigung der einzelnen PCB-Kongenere. Diss. Fachbereich Biologie Univ. Hannover.

Denker, E., Becker, P. H., Beyerbach, M., Büthe, A., Heidmann, W. A., Staats de Yanés, G. (1994) Concentrations and metabolism of PCBs in eggs of waterbirds on the German North Sea coast. Bull Environ Contain Toxicol 52:220-225.

DFG (1988) Polychlorierte Biphenyle: Bestandsaufnahme über Analytik, Vorkommen, Kinetik und Toxikologie. VCH Weinheim, Germany.

Duinker, J.C. (1986) The role of small, low density particles on the partition of selected PCB congeners between water and suspended matter (North Sea Area). Neth J Sea Res 20:229-238.

Falkner, R., Simonis, W. (1982) Polychlorierte Biphenyle (PCB) im Lebensraum Wasser (Aufnahme und Anreicherung durch Organismen- Probleme der Weitergabe in der Nahrungspyramide). Arch Hydrobiol Ergebn Limnol 17:1-74.

Focardi, S., Leonzio, C., Fossi, C. (1988) Variation in polychlorinated biphenyl composition in eggs of mediterranean water birds in relation to their position in the food chain. Environ Pollut 52:243-255.

Heidmann, W.A. (1986) Isomer specific determination of polychlorinated biphenyls in animal tissues by gas chromatography mass spectrometry. Chromatographia 22:363-369.

Larsson, P., Okla, L., Collvin, C. (1993) Reproductive status and lipid content as factors in PCB, DDT and HCH contamination of a population of Pike *(Esox lucius).* Environ Toxicol & Chem 12:855-861.

Moreira, F. (1995) The winter feeding ecology of Avocets *Recurvirostra avosetta* on intertidal areas. II. Diet and feeding mechanisms. Ibis 137:99-108.

Pavlou, S. P., Dexter, R.N. (1979) Distribution of polychlorinated biphenyls (PCB) in estuarine ecosystems. Testing the concept of equilibrium partitioning in the marine environment. Environ Sci Technol 13:65-70.

Safe, S., Hutzinger, O. (1987) Polychlorinated biphenyls (PCBs): Mammalian and environmental toxicology. In: Environmental Toxin Series 1, Springer NY.

Subramanian, A. N., Tanabe, S., Hidaka, H., Tatsukawa, R. (1987) Gain and loss rates and biological half-life of PCBs and DDE in the bodies of Adelie Penguins. Environ Pollut 43:39-46.