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Chlorinated Aromatic Hydrocarbons in the Brains and Lipids of Sparrows *(Passer domesticus* and *Passer montanus)* from Rural and Suburban Areas near Warsaw

J. Pinowski,[1] A. Niewiadöwska,[2] Z. Juricová,[3] I. Literák,[4] J. Romanowski[1]

[1] Department of Vertebrate Ecology, Institute of Ecology PAS, Dziekanów Lesny, 05-092 Lomianki, Poland
[2] National Veterinary Research Institute, Department of Pharmacology and Toxicology, Al. Partyzantów 57, 24-100 Pulawy, Poland
[3] Institute of Vertebrate Biology ASCR, Klásterní 2, 691 12 Valtice, Czech Republic
[4] Department of Infectious Diseases and Veterinary Epidemiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic

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A detailed history of the use of aromatic hydrocarbons in Poland is given in our earlier paper (Niewiadowska et al. 1998). DDT had been withdrawn from use in the country by 1975. In turn, however, limitations and then a ban on the use of Lindane (γ-HCH) were only introduced in the period 1980-1989 (Ludwicki and Góralczyk 1994). In Poland, concentrations of DDT and its derivatives in animal tissues have declined 10-20 fold since 1975 (Niewiadowska 1996, Niewiadowska et al. 1996), but are still widely present in the tissues of livestock and wild animals, as well as in human fat and milk (Falandysz 1994, Karolewski et al. 1991, Pinowski et al. 1995, Niewiadowska and Zmudzki 1996). It is not known what amounts of PCBs are brought into or produced within the country (Falandysz et al. 1992), but their concentrations in animal tissues and products in Poland are generally low and declining steadily (Niewiadowska et al. 1995).

The aim of the work described here was thus to study the contamination with organochlorine pesticides and PCBs of House Sparrows *(Passer domesticus)* and Tree Sparrows *(P. montanus),* as indicators of contamination by these xenobiotic compounds in rural and suburban areas near Warsaw. A further aim was to verify earlier results (Niewiadowska et al. 1998) suggesting that concentrations of PCBs in the sparrows in the period 1994-1996 were only one-hundredth of those noted in the years 1988-1989.

MATERIALS AND METHODS

Work was done in 1994, 1995 and 1996, near Warsaw (52°22'N, 20°52'E), in a rural area (Kepa Kielpinska, KK, and Kielpin, KL) and a suburban area (Dziekanów Lesny, DzL). DzL is a typical suburban area of Warsaw with residential (single-family) premises providing services and workshops, while the rural areas (KK and KL) are characterised by the presence of small farms among fields, though in KL these border onto a typical suburban area. The fields in the rural area include many rows of pollarded willows *(Salix* sp.). Between the 1950s and 1975, the whole study area was treated with DDT in an effort to combat the Colorado beetle *(Leptinotarsa decemlineata).* This was also true of DzL, which was still a small village surrounded by potato fields in that period. In the 1980s, the areas remaining rural (KK, KL) that were treated with organochlorine pesticides were mainly given Lindane in the forms Gammatox and Gammakarbotox. A detailed description of the study area is given in Pinowski (1967, 1968) and Pinowski et al. (1995).

The detailed subjects of investigation were the contents of organochlorine pesticides and polychlorinated biphenyls in lipid fractions extracted using Soxhlet's Method from the whole bodies of sparrows (expressed as ppm. lipid basis) and from their brains (expressed as ppm. fresh weight).

The sparrows were mist-netted in autumn and winter 1994, 1995 and 1996 and were thus

Correspondence to: J. Pinowski

juveniles, less than one-year of age, or adults greater than one-year of age. Following killing, they were stored at -20°C until analysed. The carcass dry masses and lipid contents were determined for 48 House Sparrows (15 taken at DzL on January 6 1995 and 33 taken at KK: 3 on December 15 1995, 14 on February 2 1996 and 16 on February 14 1996). Fresh-weight concentrations of chlorinated hydrocarbons (pesticides and PCBs) were in turn analysed in the brains of 51 House Sparrows (22 taken from DzL on October 26 1994, 10 from KL on December 2 1994 and 19 from KK on February 14-l5 1996) as well as 15 Tree Sparrows (taken from KL on December 2 1994).

Levels of chlorinated hydrocarbons were determined by capillary gas chromatography, with PCBs being extracted from the sample matrix using hexane (lipids) or hexane and acetone (brains). The extract was cleaned on the basis of sulphuric acid treatment (H_2S0_4 + H_2SO_4 x 25%SO_3), while separation of PCBs from organochlorine pesticides was achieved using alkaline hydrolysis (10% KOH in ethanol). Cleaned-up and concentrated extracts were analysed quantitatively and qualitatively in gas chromatographs equipped with electron-capture detectors and capillary columns. The concentrations of total PCBs were estimated by comparing selected main peak areas or heights in samples with standards (Aroclor 1260, 1254 or others).

The precision (repeatability and reproducibility) and accuracy of the methods used was validated as part of a laboratory quality assurance programme, and the limits to the determinations were found to be between 0.001 and 0.005 ppm. depending on the compound, while recoveries exceeded 90%.

The statistical processing of the material involved two-way ANOVA, with the factors taken into account being area and sex. The significance of differences between the areas were studied by applying a 95%-confidence range test, as well as by analysis of variance confined to birds from KK and KL. Unfortunately, the material did not allow for any separation of the effects of area and time of study. The material deriving from Tree Sparrows, which were not sexed and all caught at one place and time, was compared using a Student t-test with material from House Sparrows caught at the same time and place. In the case of the House Sparrows captured at DzL (for which the age was determined), an additional analysis of variance was carried out considering age and sex as factors.

RESULTS AND DISCUSSION

The lipids of 33 House Sparrows from the rural area [KK] and 15 from the suburban area [DzL] were found to contain various residues, singly or in combination, with the full list comprising HCB, α-HCH, γ-HCH, p,p'-DDE, p,p'-DDD, p,p'-DDT and PCBs (Table 1). In relation to the noted contents of pesticides and PCBs recorded in lipids, the samples were divided into 3 groups: 1) samples with no residues of organochlorine compounds, 2) samples with concentrations below the maximum residue limit (MRL) and 3) samples with concentrations higher than the MRL. The values for the MRL were greatest residual concentrations permissible in Polish law, which were taken from *Dziennik Ustaw* no. 43, item 273 from 1997, while in the case of PCBs, the proposed permissible value of 0.5 ppm. was adopted.

HCB was present at concentrations below the MRL in all samples. α-HCH also occurred at sub-MRL concentrations only, and was not recorded at all in more than half of the samples. γ-HCH was only found in one female in the suburban area, at a concentration below the MRL, but was noted in three-quarters of the individuals studied from the agricultural areas, though again at sub-MRL concentrations. p,p'-DDE residues were detected in all the birds studied, and concentrations exceeded the MRL in all the suburban birds and in almost three-quarters of those from agricultural areas. p,p'-DDD and p,p'-DDT residues were present in all samples from the suburban area and in most

samples from the agricultural areas, albeit at sub-MRL levels. Since p,p'-DDE occurred at much higher concentrations than p,p'-DDD and p,p'-DDT — as the dominant component of Σ-DDT, the distribution of the latter was the same as for p,p'-DDE. PCBs were found in all samples, but the MRL was only exceeded in 2 females from agricultural areas.

The mean concentrations of HCB, p,p'-DDE and p,p'-DDD in lipids were greater in birds from the suburban area than from the true rural areas. Differences between the areas were not significant where p,p'-DDT, α-HCH and PCBs were concerned. Σ-DDT behaved in the same way as p,p'-DDE, while the concentrations of γ-HCH were on average higher in the rural area than in the suburban area (Table 1).

All the studied samples of lipids from House Sparrows were found to contain PCB congeners. The total for all the analysed congeners amounted to ca. two-thirds of the amount of total PCBs determined and was strongly correlated with it (R^2=93%). Congeners PCBs 28, 105 and 157 were obtained from c. 30% of samples, congener PCB 156 in 44%, congener PCB 101 in 67% and congeners 183 and 206 in more than 90%. Congeners PCBs 118, 138, 153, 170, 187, 194 and 201 were present in all of the extracts, while the highest concentrations were noted for 153, 138 and 180 (Fig. 1). Significant differences between the suburban and rural areas were only found for the PCB 28 congener, which did not occur in sparrows from the former (2-way ANOVA, F=10.05, p=0.002) and 201, whose concentrations were ca. 50% higher in sparrows from the suburban area (2-way ANOVA, F=6.392, p=O.O15).

The brains of House Sparrows were not found to contain measurable amounts of either HCB or α– and γ-HCH residues. Among the DDT derivatives, only p,p'-DDE and p,p'-DDD residues were recorded. The p,p'-DDE residues were noted in all brains, though at mean concentrations that were higher in the brains of sparrows from the suburban area, as opposed to the rural areas, and higher in those from KL than KK. p,p'-DDD residues were confined to the brains of sparrows from the suburbs. Residues of total PCBs were not detected at all in the brains of sparrows from KK - the agricultural area farthest from the suburbs, and were very low in those from the other areas, with differences between them not achieving statistical significance (Table 2).

26 samples of the brains of House Sparrows (51% of the total) were not found to contain PCB congeners. The remaining samples yielded generally low concentrations of the congeners PCB 28, 44, 52, 101, 138, 153, 180, 187 and 194, with most occurring in some samples only (Figure 1). Samples from DzL birds which contained congeners most often had PCB 153, while PCBs 44 and 187 were present most frequently in the brains of birds from agricultural areas.

In the cases of the 15 Tree Sparrows, all brains were found to contain p,p'-DDE residues (at concentrations between 0.018 and 0.168 ppm., mean 0.050, SD=0.041). PCB residues were only noted in one brain, at a concentration of 0.009 ppm.. Residues of other chlorinated hydrocarbons were not found from the brains of Tree Sparrows, and a general comparison with the brains of 10 House Sparrows caught at the same time in the same place (KL) yielded no significant differences between the two in concentrations of p,p'-DDE and PCBs (Table 2, Student t-test: t= -1.0794 for DDE, 0.9434 for PCBs). Only 3 of the 10 House Sparrows were found to have PCBs in their brains.

Ten (67%) of the samples of brains from Tree Sparrows were not found to contain PCB congeners. Congener PCB 153 was recorded in one individual, and the congeners PCBs 28 and 52 in two, albeit with one additionally having PCB 44 and the other PCB 187. The latter was noted in the remaining two, of which one also contained PCB 101. Four of the brains from which PCB congeners were detected were not shown to have measurable concentrations of total PCBs.

Table 1. Chlorinated aromatic hydrocarbons *in* House Sparrow *(Passer domesticus)* fat (ppm. lipid fraction)

Area and date	sex	N		HCB	α-HCH	γ-HCH	pp'-DDE	pp'-DDD	pp'-DDT	Σ-DDT	total PCBs
Suburban (DzL) Jan. 6 1995	m	3	mean± SD	0.011±0.003	0.002±0.002	not detected	15.976±6.099	0.043±0.010	0.033±0.017	16.052±6.083	0.242±0.075
			min - max	0.008-0.014	0.000-0.004		10.298-24.44	0.036-0.058	0.018-0.057	10.413-24.50	0.157-0.339
	f	12	mean± SD	0.007±0.004	0.000±0.000	0.000±0.001	12.506±7.321	0.056±0.049	0.033±0.033	12.595±7.357	0.232±0.092
			min - max	0.002-0.014	0.000-0.001	0.000-0.003	1.680-25.56	0.020-0.196	0.004-0.129	1.751-25.60	0.128-0.410
	both	15	mean± SD	0.008±0.004	0.000±0.001	0.000±0.001	13.200±7.228	0.053±0.044	0.033±0.030	13.287±7.253	0.234±0.089
			min - max	0.002-0.014	0.000-0.004	0.000-0.003	1.680-25.56	0.020-0.196	0.004-0.129	1.751-25.60	0.128-0.410
Rural (KK) Dec. 15 1995 - Feb. 15 1996	m	15	mean± SD	0.004±0.002	0.003±0.005	0.002±0.001	3.192±3.459	0.012±0.009	0.016±0.012	3.221±3.466	0.213±0.097
			min - max	0.002-0.008	0.000-0.021	0.000-0.006	0.462-12.60	0.000-0.026	0.000-0.054	0.474-12.64	0,101-0.481
	f	18	mean± SD	0.003±0.001	0.000±0.001	0.001±0.001	2.507±2.244	0.007±0.007	0.028±0.041	2.542±2.246	0.285±0.298
			min - max	0.002-0.005	0.000-0.002	0.000-0.002	0.756-9.288	0.000-0.023	0.008-0.170	0.771-9.307	0.079-1.263
	both	33	mean± SD	0.003±0.001	0.001±0.004	0.001±0.001	2.819±2.881	0.009±0.008	0.023±0.031	2.851±2.886	0.252±0.232
			min - max	0.002-0.008	0.000-0.021	0.000-0.006	0.462-12.60	0.000-0.026	0.000-0.170	0.474-12.64	0.079-1.263
ANOVA	Area & date effect			***	NS	**	***	***	NS	***	NS
	Sex effect			**	*	NS	NS	NS	NS	NS	NS

DzL — Dziekanów Leśny, KK — Kępa Kiełpińska; m — males, f— females
ANOVA: NS — not significant, * — $p<0.05$, ** — $p<0.01$, *** — $p<0.001$
Σ-DDT = pp'-DDE + pp'-DDD + pp'-DDT

Table 2. Chlorinated aromatic hydrocarbons in House Sparrow *(Passer domesticus)* brains (ppm. fresh weight)

Area and date	sex	age	N		pp'-DDE	pp'-DDD	Σ-DDT	total PCBs
Suburban DzL Oct. 26 1994	m	juv	6	mean± SD min - max	0.532 ± 0.458 0.036 - 1.322	0.007 ± 0.006 0.000 - 0,015	0.540 ± 0.463 0.036 - 1.336	0.004 ± 0.003 0.000 - 0.008
		ad	3	mean± SD min - max	0.490 ± 0.335 0.042 - 0.849	0.009 ± 0.008 0.000 - 0.019	0.499 ± 0.343 0.042 - 0.868	0.006 ± 0.004 0.000 - 0.010
		both	9	mean± SD min - max	0.518 ± 0.421♣ 0.036 - 1.322	0.008 ± 0.007 0.000 - 0.019	0.526 ± 0.427 0.036 - 1.336	0.005 ± 0.004 0.000 - 0.010
	f	juv	7	mean± SD min - max	0.139 ± 0.119 0.011 - 0.345	0.001 ± 0.004 0.000 - 0.010	0.140 ± 0.121 0.011 - 0.345	not detected
		ad	6	mean± SD min - max	0.347 ± 0.142 0.116 - 0.527	0.003 ± 0.005 0.000 - 0.013	0.350 ± 0.144 0.116 - 0.527	0.004 ± 0.003 0.000 - 0.007
		both	13	mean± SD min - max	0.235 ± 0.166♣ 0.011 - 0.527	0.002 ± 0.004 0.000 - 0.013	0.237 ± 0.168 0.011 - 0.527	0.002 ± 0.003 0.000 - 0.007
Rural KŁ Dec. 2 1994	m		6	mean± SD min - max	0.085 ± 0.057♣ 0.028 - 0.183	not detected	0.085 ± 0.057 0.028 - 0.183	0.002 ± 0.002 0.000 - 0.005
	f		4	mean± SD min - max	0.051 ± 0.031♣ 0.017 - 0.095	not detected	0.051 ± 0.031 0.017 - 0.095	0.001 ± 0.002 0.000 - 0.005
Rural KK Feb. 14 1996	m		10	mean± SD min - max	0.021 ± 0.014♣ 0.006 - 0.053	not detected	0.021 ± 0.014 0.006 - 0.053	not detected
	f		9	mean± SD min - max	0.016 ± 0.007♣ 0.008 - 0.032	not detected	0.016 ± 0.007 0.008 - 0,032	not detected
ANOVA according to area and date	Area & date effect				**	**	**	NS
	Sex effect				*	*	*	NS
ANOVA sparrows from DzL only	Age effect				NS	NS	NS	*
	Sex effect				*	*	*	*

♣ — Though, according to 95% confidence range test, there is no significant difference in DDE level between the both rural areas (KŁ and KK), the area effect is still significant, if birds from Dziekanów Leśny, Oct. 26, 1994 are excluded from the ANOVA.

juv — juvenile, ad — adult, KŁ — Kiełpin. Other codes as in Table 1.

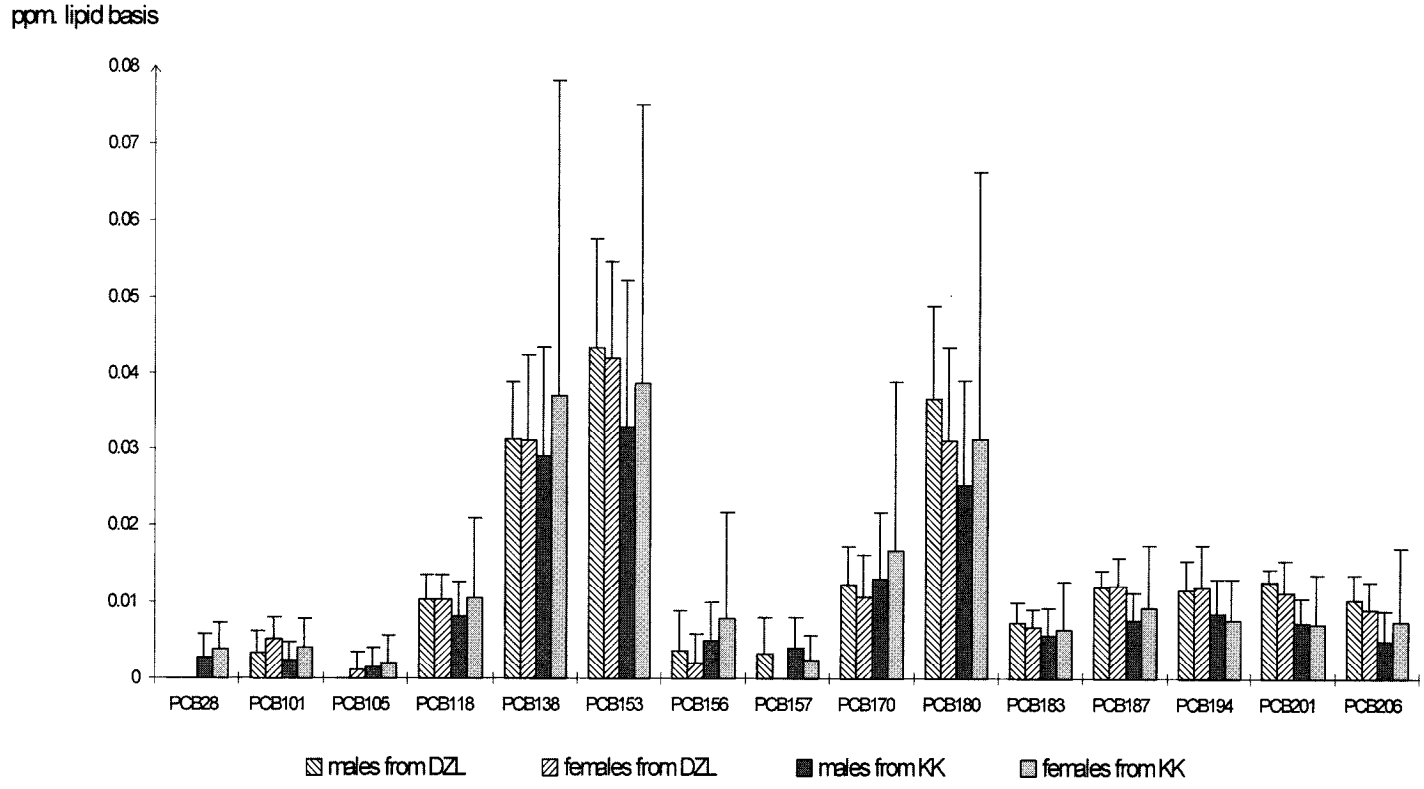

Figure 1. PCB congeners in the lipids of House Sparrows *Passer domesticus* (mean and standard deviation).
DZL - Dziekanów Lesny, KK - Kepa Kielpinska

The concentrations of all pesticides were greater in the lipid of males from a given area, as opposed to females, though the difference only achieved statistical significance for HCB and α-HCH. Concentrations of PCBs were similar in the two sexes (Table 1). In brains too, the mean concentration of p,p'-DDE was found to be between 1.5 times and twice as great in males from a given area as in females, and in this case the difference was statistically significant. Since concentrations of p,p'-DDE residues are several tens of times greater than those of p,p'-DDD, what applies to the former is also true of Σ-DDT. A significant effect of sex was also recorded for p,p'-DDD residues in the brains of House Sparrows. In this case too, the mean concentration was higher in males than in females (Table 2). However, the effect of sex was not significant for PCBs, although mean concentrations were again higher in males from a given area than in females.

In relation to the known ages of the birds caught in DzL in autumn 1994, the concentrations of p,p'-DDE (and hence Σ-DDT), p,p'-DDD and PCBs in brains were found to be significantly greater in males than in females in both young and old birds. However, the effect of age was not significant for any of these derivatives of DDT. Both sex and age exerted significant effects in the case of PCBs, since these were not discovered at all in young females (Table 2).

When compared with those from agricultural areas, House Sparrows from the suburban area (DzL) were found to have concentrations of p,p'-DDE that were several times higher in lipid and even several tens of times higher in brains. In turn, the brains of sparrows from KL (where the fields abut onto suburban construction) had concentrations of this compound that were 3-4 times higher than those from the purely-agricultural KK (Tables 1 and 2). The higher concentrations of DDT derivatives in the suburban area may be explained by the fact that the period before the onset of building in the 1970s and 80s saw this area subject to the cultivation of potatoes and hence to intensive measures to combat the Colorado beetle using DDT (azotox). Equally, the possibility cannot be excluded that DDT was used illegally in gardens after 197.5. Lindane (γ-HCH) was used by farmers in the form of Gammatox and Gammakarbotox up to the end of the 1980s. This accounts for its greater concentrations in the tissues of House Sparrows from agricultural, as opposed to suburban, areas (Table 1).

Both brains and body lipids were shown to have higher mean concentrations of pesticides if from the male birds of a given area, as opposed to the females (Tables 1 and 2). Many other authors (e.g. Lemmetyinen et al. 1982, Ohlendorf and Miller 1984) have given higher concentrations of derivatives of DDT and PCBs in male birds than in females. The effect has usually been explained by reference to the excretion of lipid-soIuble toxins together with eggs, as has been well-documented in literature (Becker et al. 1989). However, we also observed the phenomenon among birds in the first year of life, for whom the aforementioned mechanism cannot apply. The effect was particularly clear for PCBs, which were not present in measurable quantities in the brains of young females, but present at similar frequency and in similar amounts in young males and adult birds of both sexes (Table 2). Thus a role would seem to be played by other factors like differences in the contamination of food and the excretion of toxins via other pathways.

The present work confirms our earlier findings (Niewiadowska et al. 1988) that concentrations of PCBs in House Sparrows and Tree Sparrows from the study area in the period 1994-1995 were less than one-hundredth of those noted in the years 1988- 1989 (e.g. respectively 0.005 as opposed to 2.739 and 0.001 as opposed to 2.797 ppm. fresh weight in brains).

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