Bull. Environ. Contam. Toxicol. (1992) 48:828–834

Environmental Contamination and Toxicology

Polychlorinated Biphenyls in Two Species of Arctic Seabirds from the Svalbard Area

F. F. Daelemans,[1] F. Mehlum,[2] and P. J. C. Schepens[1]

[1]Toxicological Center, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium and [2]Norwegian Polar Research Institute, Rolfstangveien 12, N-1330 Oslo Lufthavn, Norway

Anthropogenic long-range pollution of the Arctic could lead to rapid environmental changes at a large geographical scale. Arctic ecosystems are regarded as very vulnerable since the energy flow is channeled through only one or a few essential links (Jensen 1990). The apparant stability and its survival over longer periods is probably due to the large spatial scale involved which provides the mending of regions of disturbance by repopulation from undisturbed areas. The large geographical scale of man-made pollution from the industrialized countries may interfere with this basic factor of stability in the Arctic ecosystem. Organochlorine compounds is one important group of pollutants transported through the atmosphere and ocean currents from the industrial areas to the Arctic. The ability of organochlorine compounds to enter the food chain in significant quantities, as a result of their relative high stability, lipid solubility and tendancy for bioaccumulation, poses a possible hazard for the Arctic fauna. In this respect polychlorinated hydrocarbons and more in particular polychlorinated biphenyls (PCBs) seem to be one of the most dangerous pollutants of the Arctic environment (Jensen 1990). The archipelago of Svalbard in the European Arctic is an area with only minor local industrial activities, and the anthropogenic pollution recorded in the area is assumed to be mainly of foreign origin. The wildlife in Svalbard is rich in numbers (Mehlum 1990) and large populations of seabirds and marine mammals constitute major parts of the fauna. The published information on the levels of organic pollutants in animals from Svalbard is rather limited. However some studies have been made on birds and mammals (Norheim 1984) showing that despite of the remote location of these prestine areas, the fauna is contaminated with considerable levels of anthropogenic pollutants. In the Glaucous Gull, *Larus Hyperboreus*, especially high levels of PCBs have been recorded (Norheim 1984). This species partly acts as a predator and during the breeding season it is preying upon other seabird's eggs and chicks, and has similar ecological function as birds of prey in other places (Mehlum 1990). Because of bioaccumulation of organochlorines one would suspect to find high levels of these substances in this species, which is the main avian predator in the Svalbard area.

In the existing literature of PCBs in seabirds from the Svalbard area only reports on the level of total PCBs are given. From a toxicological point of view, however, some congeners are more interesting than others and may account for most of the toxicity. A large portion of PCB toxicity is due to the presence of non-ortho chlorobiphenyls (Safe 1985a) having only substitution in the para and meta position and thus exhibiting maximum coplanar conformational character. The most active among them, 3,3',4,4'-tetrachlorobiphenyl (# 77) (Ballschmitter 1980), 3,3',4,4',5-pentachlorobiphenyl (# 126) and 3,3',4,4',5,5'-hexachlorobiphenyl (# 169) show toxic responses typical of the highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-T_4CDD) (Safe 1985a,b) The introduction of a single ortho-chloro substituent results in decreased coplanarity between

Send reprint requests to F.F. Daelemans at the above address.

the two phenyl rings. This change in conformation diminishes but does not eliminate the toxic effects. In this paper we present data on PCB levels in two species of Arctic seabirds from the Svalbard area. One is the Glaucous Gull and the other the Black Guillemot *Cepphus Grylle*. Concentrations of total PCB as well as those of 10 different congeners are given. Total concentrations of each homologue isomer series are also presented. Special attention is given to three of the most toxic non-ortho chlorobiphenyls (#77, #126 and #169).

MATERIALS AND METHODS

Thirteen Glaucous Gulls were caught in July 1990 at the waste disposal site of Longyearbyen, Svalbard (78°N, 15°E). They were all adult birds weighing between 1.3 and 1.9 kg. The liver was removed and kept frozen at - 25 °C until analysis. The same procedure was followed for ten Black Guillemots. They were caught along the coastline of Adventfjorden, close to Longyearbyen, where they were feeding on benthic organisms. They were all adult birds weighing between 335 and 420 g.

We used an extraction procedure based upon the one of Tanabe et al. (Tanabe 1987). A 10 g liver sample was cut into small pieces and placed in a beaker containing 400 mL 1 N ethanolic KOH and the whole was placed in an ultrasonic bath for two hours. After this saponification step, 400 mL of distilled water and 1 μg of internal standard being 3,3',4,4'-tetrabromobiphenyl were added. This crude extract was partitioned twice with 100 mL hexane. The two extracts were combined and reduced to a volume of 2.5 mL.

To eliminate the remaining lipids, we used gel permeation chromatography (GPC) (Norstrom 1986). A Biorex MP column (Bio-Rad Laboratories) with an internal diameter of 2.5 cm and a maximum bed length of 45 cm was packed with 60 g of Bio Beads SX-3, 200-400 mesh (Bio-Rad Laboratories) swollen in a mixture of hexane:dichloromethane (50:50) which gave a bed length of about 40 cm. The column was connected to an injector system with a 5 ml sample loop and on the other end connected to a UV detection cell. The column was eluted with an upward flow. The first 150 mL were discarded, the next 130 mL were collected for further use. The elution pattern was identifiable with the one obtained by Norstrom et al. (Norstrom 1986). For further cleanup steps, the extract was devided in two equal fractions, both evaporated to a volume of 2 mL hexane. One fraction was cleaned up by means of carbon chromatography and served to determine the three most toxic non-ortho congeners mentioned earlier. Active carbon from Wako Pure Chemical Industries was heated at 100 °C in an oven at reduced pressure and was stored in a desiccator. The elution column (5 mm internal diameter and 40 mm height) with a 100 mL reservoir was packed with 125 mg active carbon in hexane. The GPC eluate was added to the column and initially eluted with 100 mL of dichloromethane:hexane (20:80) at one drop per second flow rate. This fraction was rejected and elution proceeded with 100 mL ethylacetate:benzene (50:50). This second eluate was concentrated to 100 μL and made up with 5 mL hexane, and was vortexed with 5 mL 5 % fuming sulfuric acid in concentrated sulfuric acid. The two layers were centrifuged at 6000 rpm during 10 minutes. The upper hexane layer was reduced to 20 μL of which 5 μL were injected in the GC/MS. The second fraction was subjected to a Florisil cleanup procedure (Norstrom 1988) and served to determine the total PCB concentration in the liver sample. Florisil, 60-100 mesh, from Merck was deactivated with 1.2 % water and 8 g were packed with hexane in an elution column (10 mm internal diameter and 200 mm height) with a 100 mL reservoir. The GPC eluate was placed on the column which was eluted with

40 mL hexane. The eluate was reduced to 100 μL volume and 5 μL were injected in the GC/MS for final analysis.

About 5 g sample was taken (exact weight must be known) and mixed in a mortar with a 7 times higher weight Na_2SO_4. This mixture was packed onto a column and eluted with 50 % dichloromethane in hexane. After evaporating and drying, the lipid content was determined gravimetrically.

The samples were analyzed on a Hewlett-Packard 5988 A GC/MS employing the electron impact ionization mode at 70 eV using selected ion monitoring (SIM). The GC was equipped with a 60 m long x 0.25 mm internal diameter fused silica DB-5 column with a film thickness of 0.25 micron provided with a 1 m deactivated fused silica retention gap. The splitless injection mode was held for 1 min after injection at an oven temperature of 60 °C on a split/splitless injector at 250 °C. Temperature programming started with a rate of 20°C/min until 200 °C and continued at a rate of 1 °C/min until 275 °C. The carrier gas was helium and the transfer line was kept at 280 °C.

For GC/MS identification, we used a paper by Mullin et al. (Mullin 1984) describing the elution order of all 209 PCB congeners. This elution order was a guideline for the establishment of 5 SIM groups seperated by 4 group switch times, defined by # 104, # 77, # 128 and # 208, so not all ions had to be checked in every group. The retention time, the masses and the ratio of the confirmation ion intensity to the quantitation ion intensity in comparison with the expected ratio for each level of chlorination were used as the identification criteria. The average experimental relative deviation of the theoretical ratios for the chlorinated PCBs was 6.4 %. For the trichloro through the heptachloro PCB congeners, at least one ion in the $(M+70)^+$ ion cluster was examined to verify that no coeluting PCB congener containing 2 additional chlorines were present. For the dichloro through the octachloro PCB congeners, at least one ion in the $(M+35)^+$ ion cluster was examined to verify that no coeluting PCB congener containing 1 additional chlorine was present, and for all congeners starting from the dichloro, at least 1 ion in the $(M-70)^+$ ion cluster must be present. For the GC/MS quantitation of the total PCB concentration, a calibration curve was set up with nine pure PCB isomers (# 1, # 8, # 28, # 52, # 118, # 153, # 180, # 203, # 206, # 209) instead of using the commercial mixtures of Arochlors, Kanechlors or Clophens. After qualitative identification was accomplished, the quantitation ion areas of all identified congeners within each homolog isomer series were summed and the total concentration of each homolog series was calculated using the appropriate homolog respons factor. The three non-ortho congeners were quantitated using their respective pure standards for calibration.

RESULTS AND DISCUSSION

Total PCB concentrations in the liver of both Glaucous Gull and Black Guillemot are presented in table 1. The average concentration in the liver of the Glaucous Gull is 20.9 μg/(g wet weight) and about 160 times higher than the concentration in the liver of the Black Guillemot. The average lipid content is (4.2 $\pm$ 0.2) % and thus the average total PCB concentration results in 498 μg/(g lipid). It is apparant that the concentrations in the Glaucous Gull vary strongly; between 2,1 and 77,7 μg/(g wet weight) and with a relative standard deviation of 128 %. For the Black Guillemot, a significantly smaller variance is noted; between 77 and 213 ng/(g wet weight) and with a relative standard deviation of 37 %. There is no lipid content information available for the Black Guillemot.

Table 1 : Concentrations of PCBs in liver of Black Guillemot and Glaucous Gull. All concentrations are ng/(g wet weight)

	Total PCB concentration			
	Glaucous Gull		Black Guillemot	
	20950 ± 26800		128 ±47	
Congener	Absolute concentration		Relative concentration	
	Glaucous Gull	Black Guillemot	Glaucous Gull	Black Guillemot
# 77	2.6 ± 2.0	> DL*	0.03 ± 0.02	< DL
# 126	20.0 ± 27.1	0.55 ± 0.04	0.11 ± 0.04	0.34 ± 0.02
# 169	10.0 ± 17.9	0.08 ± 0.01	0.04 ± 0.02	0.051 ± 0.001
# 114	29 ± 37	0.3 ± 0.1	0.14 ± 0.06	0.20 ± 0.04
# 118	1875 ± 2460	10.1 ± 3.9	7.5 ± 2.7	7.9 ± 2.3
# 123	14 ± 15	0.18 ± 0.12	0.10 ± 0.04	0.17 ± 0.13
# 156	307 ± 438	2.2 ± 0.8	1.4 ± 0.5	1.7 ± 0.2
# 157	61 ± 78	0.69 ± 0.33	0.3 ± 0.1	0.5 ± 0.1
# 167	330 ± 467	-	1.5 ± 0.5	-
# 189	45 ± 70	0.38 ± 0.29	0.21 ± 0.08	0.25 ± 0.12

* : Below detectionlimit ** : Information not available

Table 2 shows a clear similarity between the 2 birds when their PCB pattern, expressed in terms of their isomer group composition, is compared. Figure 1 gives the composition of the PCBs in liver according to their isomer groups for both birds. For the Black Guillemot, the lower chlorinated PCBs contribute slightly more to the total PCB mass in comparison to the Glaucous Gull. Almost 55% of the total PCB concentration is represented by hexachlorobiphenyls in Glaucous Gull followed by 24% hepta- and 14 % pentachlorobiphenyls. For the Black Guillemot, these percentages are respectively 50.8 % hexa-, 23.9 % hepta- and 17.4 % pentachlorobiphenyls.

Table 2 : Relative composition (in %) of total PCBs and Arochlors according to isomer groups. Arochlor data from Kannan et al. (Kannan 1987)

Number of chlorines	Glaucous Gull	Black Guillemot	Arochlor 1262	Arochlor 1260	Arochlor 1254
1	< DL*	< DL	< DL	< DL	< DL
2	< DL	< DL	< DL	< DL	< DL
3	0.23 ± 0.19	0.6 ± 0.3	< DL	< DL	0.3
4	2.4 ± 1.0	4.1 ± 1.8	< DL	< DL	18.9
5	14.0 ± 2.8	17.4 ± 4.4	1.6	8.9	54.0
6	55.0 ± 5.3	50.8 ± 3.2	18.6	48.1	24.5
7	24.1 ± 3.9	23.9 ± 5.8	48.0	38.9	2.4
8	3.7 ± 1.3	3.2 ± 2.5	28.1	4.1	< DL
9	0.4 ± 0.1	< DL	3.7	< DL	< DL
10	0.08 ± 0.04	< DL	< DL	< DL	< DL

* : Below detectionlimit

In figure 2, this resemblence is statistically represented using a principal component analysis scatterplot. The theoretical background of this multivariate variance analysis is explained elsewhere (Sharaf 1986). In this plot, several Arochlors (their composition is also expressed according to their isomer groups) are compared with the PCB patterns in the liver of both birds. The distance between the points (each point represents an Arochlor- or liver composition) on the plot is a measure of their variance; the closer the points, the more alike the corresponding compositions are. This plot explains 95 % of the total variance between all the plotted points. From the plot, we see that all liver samples are concentrated in one spot and lie closest to Arochlor 1260.

Looking at table 1 again, the total concentration, for the Gull, of the 3 selected non-ortho congeners represents only 0.18 % of total PCB concentration. It is congener # 126 with the highest average concentration (0.11 ± 0.04) % followed by # 169 % (0.04 ± 0.02) % and # 77 (0.03 ± 0.02) %. In the individual liver of the Black Guillemot, the non-ortho PCB concentrations were below the detectionlimit of 0.5 ppb. For this reason pooled samples were taken. Again, PCB #126 has the highest concentration of the 3 non-ortho PCBs.

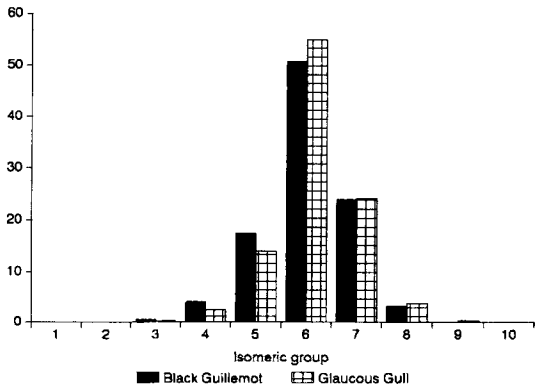

Figure 1 : Relative composition of PCBs in liver of Black Guillemot and Glaucous Gull according to isomer group

The relative potencies of induction (T_4CDD = 1) are taken from the data of Safe et al. (Safe 1985b) who measured induction potencies of various congeners to half-maximaly induce AHH (Arylhydrocarbonhydroxylase) and EROD (Ethoxyresorufin-*O*-deethylase) in hepatoma cell line. The T_4CDD toxic equivalent levels were calculated as the actual concentration (in pmol/(g wet weight)) multiplied by the relative potency of induction (Kannan 1988a). Using this unit, table III shows the importance in toxic contribution, about 99 %, of congener # 126. It has a significantly higher value as # 77 and # 169. Measured with respect to the AHH enzyme induction, the toxic equivalent level of # 126 equals 18 pmol/(g wet weight) meaning that it exhibits the same induction effects on the AHH enzyme as 5.8 ng/(g wet weight) 2,3,7,8-T_4CDD.

Table 3 : Toxic equivalence values for non-ortho and mono-ortho PCBs present in Glaucous Gull and Black Guillemot in pmol/(g wet weight).

Congener	Glaucous Gull		Black Guillemot	
	AHH	EROD	AHH	EROD
		non-ortho		
# 77	1.9×10^{-2}	1.8×10^{-2}	< DL	< DL
# 126	$1.8 \times 10^{+1}$	$2.1 \times 10^{+0}$	4.6×10^{-1}	$1.2 \times 10^{+0}$
# 169	3.3×10^{-2}	2.1×10^{-1}	2.5×10^{-4}	1.6×10^{-3}
		mono-ortho		
# 114	6.7×10^{-3}	2.8×10^{-2}	6.1×10^{-5}	2.6×10^{-4}
# 118	3.4×10^{-2}	1.1×10^{-1}	1.8×10^{-4}	6.2×10^{-4}
# 123	7.8×10^{-4}	6.9×10^{-4}	9.9×10^{-6}	8.9×10^{-5}
# 156	3.0×10^{-2}	1.7×10^{-1}	2.0×10^{-4}	1.2×10^{-3}
# 157	1.7×10^{-2}	2.4×10^{-2}	1.9×10^{-4}	2.7×10^{-4}
# 167	5.1×10^{-3}	1.8×10^{-2}	-	-
# 189	7.3×10^{-4}	2.6×10^{-3}	3.7×10^{-6}	2.2×10^{-5}

An attempt was made to determine the dioxin levels; all results were below our limit of detection. It does learn us that the non-ortho PCBs are a greater toxic threat than the dioxins and furans; an analogue statement has been reported earlier based on the results from human adipose tissue samples (Kannan 1988b).

The concentration of some of the mono-ortho substituted PCBs is much higher compared to the 3 non-ortho congeners. Again, calculating the toxic equivalent level of some of the most toxic mono-ortho PCBs, we see in table 3 that these values are of the same order of magnitude as # 77 and decrease gradually. They do not contribute significantly to the total toxicity but they cannot be neglected during the analysis.

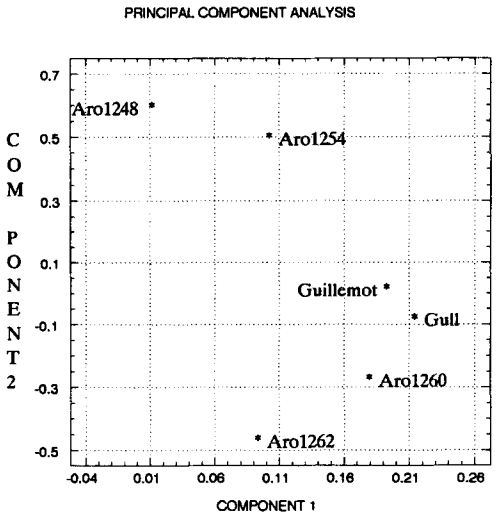

Figure 2 : Principal component analysis of the liver extracts of Black Guillemot and Glaucous Gull. Datapoints are represented as composition according to isomer group.

Expressing the concentrations of the non-ortho PCBs as total PCB normalized concentrations, comparisons can be made with concentrations of the same congeners in commercial mixtures (Borlakoglu 1990) or biological samples from a lower trophic level. In figure 2, we could see that the PCB pattern of the bird samples resemble most Arochlor 1260. Kannan et al. (Kannan 1987) reported the concentrations of the non-ortho and mono-ortho PCB congeners in Arochlor 1260. The resulting selective enrichment factors (SEF) are thus calculated by deviding the total PCB normalized concentration of the PCB of interest in the biological sample by the total PCB normalized concentration of the same PCB in Arochlor 1260. Congener #126 is 210 times enriched and #169 has a SEF greater than 4400. Congener # 77 has a SEF of 1 meaning there was no selective enrichment.

REFERENCES

Balschmitter K., Zell M. (1980) Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Fresenius' Z. Anal. Chem. 302:20-31

Borlakoglu J.T., Wilkins J.P.G., Walker C.H., Dils R.D. (1990) Polychlorinated biphenyls in extracts of brain from manx shearwaters. Bull. Environ. Contam. Toxicol. 45:819-823

Jensen J. (1990) State of the Arctic Environment : Report on Chlorinated Organics. Draft Report for the Finnish Initiative Meeting, April 17-24 1990. Technical background paper of indian and northern affairs Canada.

Kannan N., Tanabe S., Wakimoto T., Tatsukawa R. (1987) Coplanar polychlorinated biphenyls in Arochlor and Kanechlor mixtures. J. Assoc. Offic. Anal. Chem. 70:451-454

Kannan N., Tanabe S., Tatsukawa R. (1988a) Toxic potential of non-ortho and mono-ortho PCBs in commercial PCB preparations: 2,3,7,8-T_4CDD toxicity equivalence factors approach. Bull. Environ. Contam. Toxicol. 41:267-276

Kannan N., Tanabe S., Tatsukawa R. (1988b) Potentially hazardous residues of non-ortho chlorine substituted coplanar PCBs in human adipose tissue. Arch. Environ. Health. 43(1):11-14

Mehlum F. (1990) Birds and mammals of Svalbard. Polarhandbook 5. Oslo, Norsk Polarinstitutt.

Mullin M.D., Pochini C.M., McCrindle S., Romkes M., Safe S.H., Safe L.M. (1984) High-resolution PCB analysis: Synthesis and chromatographic properties of all 209 PCB congeners. Environ. Sci. Technol. 18:466-476

Norheim G., Kjos-Hanssen B. (1984) Persistent chlorinated hydrocarbons and mercury in birds caught off the west coast of Spitsbergen. Environ. Pollut. Ser. A 33:143-152

Norstrom R.J., Simon M., Mulvihill M.J. (1986) A gel-permeation/column chromatography cleanup method for the determination of CDDs in animal tissue. Int. J. Environ. Anal. Chem. 23:267-287

Norstrom R.J., Simon M., Muir D.C.G., Schweinsburg R.E. (1988) Organochlorine contaminants in arctic marine food chains: Identification, geographical distribution, and temporal trends in polar bears. Environ. Sci. Technol. 22:10631071

Safe S., Bandiera S., Sawyer T., Robertson L., Safe L., Parkinson A., Thomas P.E., Ryan D.E., Reik L.M., Levin W., Denomme M.A., Fujita T. (1985a) PCBs: Structure-function relationships and mechanism of action. Environ. Health Perspect. 61:47-56

Safe S., Bandiera S., Sawyer T., Zmudzka B., Mason G., Romkes M., Denomme M.A., Okey B., Fujita T. (1985b) Effects of structure on binding to the 2,3,7,8-TCDD receptor protein and AHH induction - halogenated biphenyls. Environ. Health Perspect. 61:21-23

Sharaf M.A., Illman D.L., Kowalski B.R. (1986) Chemometrics. John Wiley & Sons, New York.

Tanabe S., Kannan N., Wakimoto T.,Tatsukawa R. (1987) Method for the determination of three toxic non-orthochlorine substituted coplanar PCBs in environmental samples at part-per-trillion levels. Int. J. Environ. Anal. Chem. 29:199-213

Received August 5, 1991; accepted December 10, 1991.