Bull. Environ. Contam. Toxicol. (1990) 45:552–559

Bulletin of Environmental Contamination and Toxicology

Distribution of Polychlorobiphenyls and Hexachlorobenzene in Different Tissues of the Dab (*Limanda limanda* L.) in Relation to Lipid Polarity

Ulrike Kammann, Rainer Knickmeyer, and Hans Steinhart

Institut für Biochemie und Lebensmittelchemie, Universität Hamburg
Grindelallee 117, 2000 Hamburg 13, FRG

Cyclic organochlorines are environmentally persistent and widely distributed in marine ecosystems. Fish can accumulate these substances from water and from their food (Bruggeman et al. 1981; Boon 1984). There is a growing interest in predicting the bioaccumulation of these components in aquatic organisms. Only little is known of the distribution of single polychlorobiphenyl (PCB) congeners in fish with the exception of some laboratory experiments (Boon 1984; Solbakken et al. 1984). It was shown in previous studies that hexachlorobenzene (HCB) and PCB transfer from female livers to ovaries causes significant alterations in organochlorine contamination and PCB patterns in dab (Knickmeyer and Steinhart 1989).

The aim of the present study is:
- to investigate patterns of 26 individual PCB congeners and HCB in different organs of female dabs (*Limanda limanda L.*)
- to determine the influence of total lipid content as well as polar lipid fraction and neutral lipid fraction on the distribution of PCB congeners in the tissues.

MATERIALS AND METHODS

The dabs were collected at 17 stations during each of 5 cruises between December 1988 and May 1989 in the German Bight:
- December cruise of the FS VALDIVIA from 28.11.-10.12.88
- January cruise of the FS VALDIVIA from 19.01.-25.01.89
- February cruise of the FS GAUSS from 27.02.-03.03.89
- April cruise of the MS SENKENBERG from 10.04.-13.04.89
- May cruise of the FS GAUSS from 02.05.-07.05.89.

The animals were obtained by beam-trawling, then washed with sea water, wrapped in aluminium foil (pre-cleaned with

Send reprint requests to H. Steinhart at the above address.

acetone and n-hexane) and immediately frozen and stored at -25°C. After determination of length and sex, liver, gills, ovaries, kidney, gallbladder and stomach with content were removed. The organs of 10 individual fish were pooled separately for every station, and lyophilized. Only sexual mature, female individuals (bodylength > 18cm) were used for analysis (Htun-Han 1978 a, b). The tissues were ground in an agate mortar with sea sand and Na_2SO_4 and extracted with n-hexane in a Soxhlet apparatus for 8hr. In order to separate the lipids from the extracts, an Al_2O_3-column was used. The extracts were shaken with H_2SO_4 and transferred onto a SiO_2-column. The organochlorines were eluted with n-hexane. The method has been described in detail by Knickmeyer and Steinhart (1989).

A Carlo Erba HRGC 5300 gas chromatograph equipped with a AS 550 on-column autosampler and ^{63}Ni-ECD (300°C) was used for the detection of the organochlorines. The column for GC-anlysis was a 50m SE 54 fused silica column with 0.32mm ID and a 0.25 micron film. The carrier gas was helium (2ml/min). The make-up gas was argon-methane 90/10 (30ml/min). The temperature program used started with 70°C, increasing 40°/min up to 150°C, followed by an increase of 4°/min up to 280°C. Isothermal runs at 280°C oven temperature were employed for 12 min. A Merck-Hitachi 2000 integrator was used for peak identification and quantification. ϵ-Hexachlorocyclohexane (ϵ-HCH) was the internal standard. Only the congeners which elute as well separated peaks from the capillary column were considered in comparing the different PCB patterns (Duinker and Hillebrand 1983). The standard mixture contained 26 individual PCB congeners (Fig. 1, numbering according to Ballschmiter and Zell (1980)), HCB and ϵ-HCH. Detection limits for most organochlorines were in the 0.5-10 ng/g lipid range.

Tissue samples were extracted with chloroform/methanol according to the method of Bligh and Dyer (1959) in order to determine the lipid composition. The extracts were reduced nearly to dryness in a rotary evaporator. The extracted lipids were subsequently air-dried at 65°C for 4hr in an oven. The content of total lipids was determined gravimetrically. Total lipids (200-500mg) were dissolved in 25ml n-hexane and extracted three times with 20ml methanol/water (90/10) according to Schneider (1980). A polar, methanol-soluble and a neutral lipid fraction remaining in the n-hexane were obtained and subjected to gravimetrical analysis. The polar lipid fraction contained the phospholipids, whereas free fatty acids, acylglycerids and cholesterols remained in the neutral fraction (Schneider 1980). This method of lipid class characterisation is suitable for clarifying the influence of lipid polarity

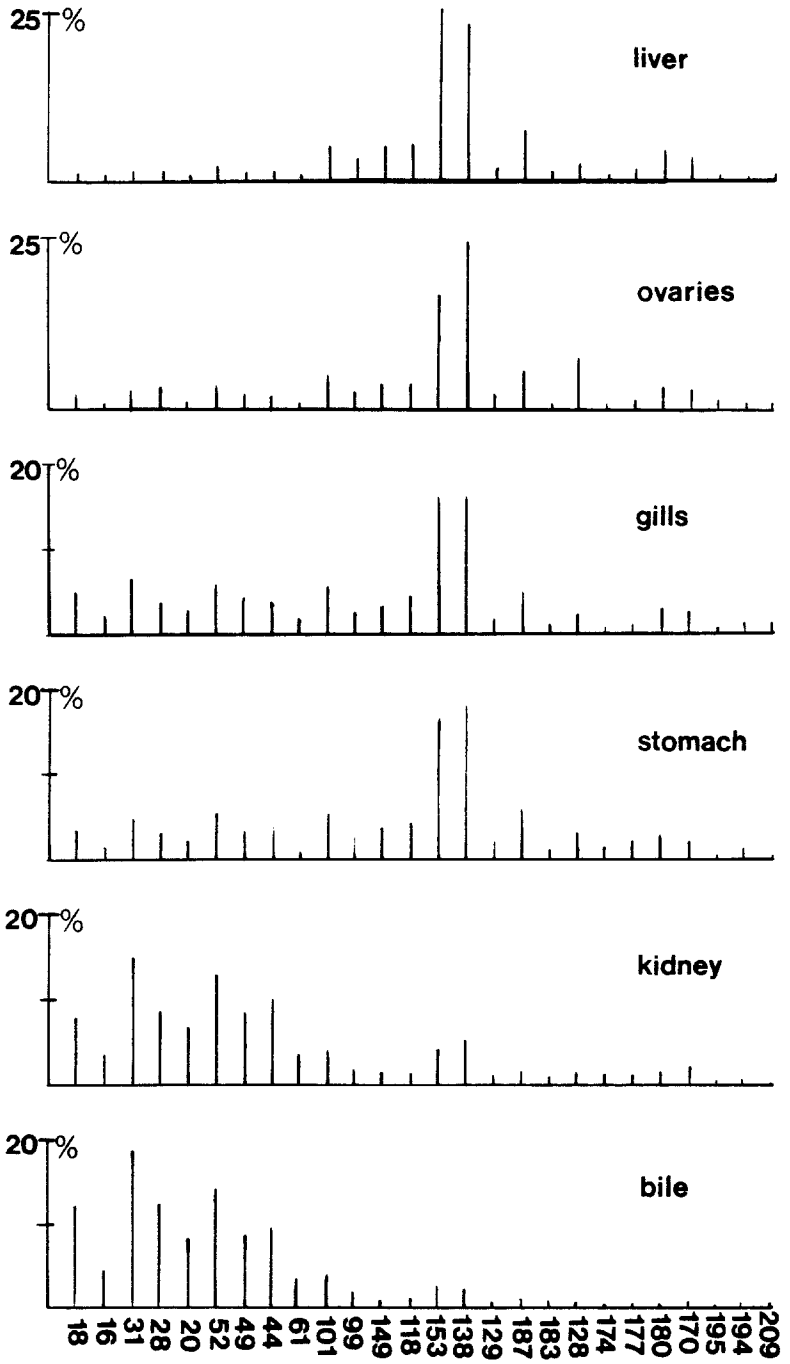

Figure 1. PCB patterns in organs of dabs (sampling position: 54°30'E 8°13'N, January 1989, 10 individuals pooled). The contribution of each PCB congener to the sum of the 26 congeners is given in order of the elution from the GC column.

on PCB distribution in dab tissues.

RESULTS AND DISCUSSION

All tissues under investigation exhibited a distinct PCB pattern, independent of sampling station and season. Fig. 1 shows the contribution of each PCB congener to the sum of the 26 congeners under investigation (Σ-PCB) for all tissues. PCB patterns of kidney and gallbladders show large contributions of tri- and tetrachlorobiphenyls. High amounts of polar lipids in kidneys and gallbladders correlate to increased contributions of more polar tri- and tetrachlorobiphenyls (Tab. 1). Patterns of liver, ovaries, gills and stomach, however, were dominated by penta- and hexachlorobiphenyls, PCB 153 and PCB 138. In these tissues large amounts of penta- and hexachlorobiphenyls correlate with high amounts of neutral lipids (Tab. 1). PCB patterns of stomach reflect the actual food composition of the dab. The PCB patterns of ovaries were directly influenced by transfer of liver lipids with a preferred transfer of PCB 138 and 128 from livers to ovaries (Knickmeyer and Steinhart 1989).

Duinker and Hillebrand (1983) reported that PCB patterns of water were dominated by lower chlorinated congeners. Several authors report the high efficiency of the uptake of the lower chlorinated biphenyls by fish from water and food (Bruggeman et al. 1981; Boon 1985). The direct uptake of higher chlorinated biphenyls with low clearence rates from water appeared to be less efficient than from

Table 1. Lipid composition of dab tissues sampled in January 1989. 25 individual fish were randomly choosen from all 17 stations and pooled. Total lipids: percentage of total lipids according to Bligh and Dyer (1959) related with the dry weight. Neutral lipids: n-hexane soluble lipid fraction expressed as percentage of total lipids. Polar lipids: methanol soluble lipid fraction expressed as percentage of total lipids.

tissue	total lipids	polar lipids	neutral lipids
liver	49.0	36.5	63.5
gills	14.3	45.7	54.3
ovaries	19.9	71.9	28.2
stomach	23.8	78.5	21.5
kidney	20.3	85.6	15.4
gallbladder	38.0	94.4	5.6

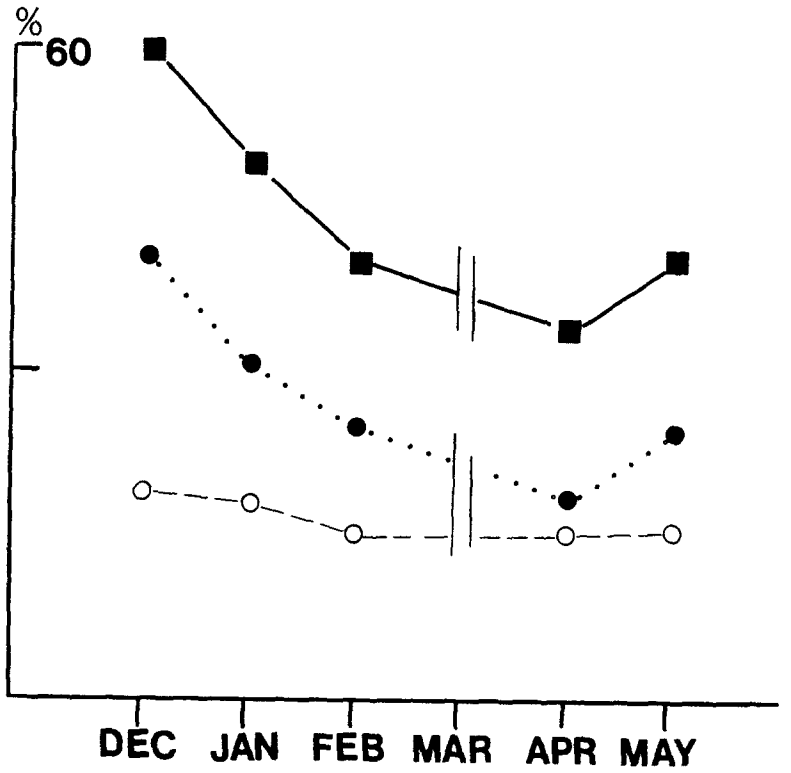

Figure 2. Variation in lipids related to dry matter in female dab livers (sampling position : 54°30'E 8°13'N, December 1988 - May 1989, 10 individuals pooled).
■= total lipids (chloroform/methanol extractable),
○= polar lipids, ●= neutral lipids.

contaminated food (Bruggeman et al. 1984; Thormann 1989). The congener composition of the gill pattern shows no direct influence of equilibration with the surrounding water. The gill lipids, however, exhibited a composition comparable to the liver with a great contribution of neutral lipids. These results suggest that lipid composition is more important for determining congener distribution in dab gills than equilibration with sea water.

The annual reproductive cycle in female dab causes a mobilization of body reserves which are partly utilized for the anabolism of the eggs. An increase in the gonosomatic index during the spawning season caused a decrease of the hepatosomatic index, reaching its minimum in the postspawning period (Htun-Han 1978b). Büther (1988) found a decrease of lipids in female dab livers from around

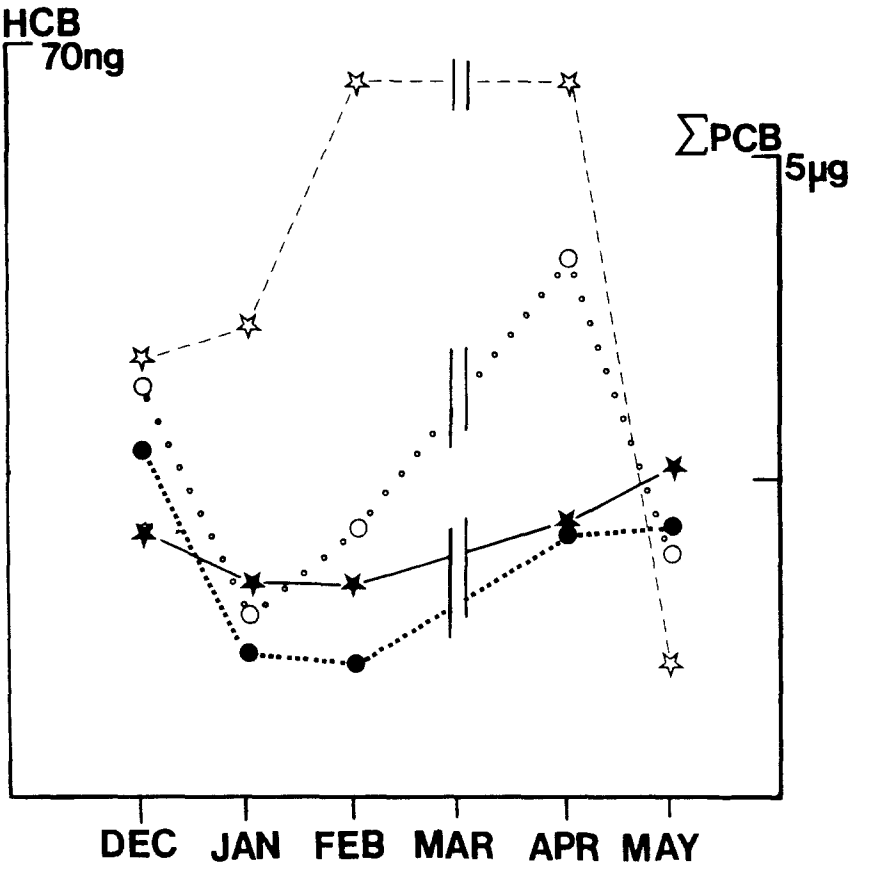

Figure 3. Variation in organochlorines related to lipids in female dab livers and ovaries (sampling position : 54°30'E 8°13'N, December 1988 - May 1989, 10 individuals pooled).
●= Σ-PCB in female livers, ○= Σ-PCB in ovaries,
★= HCB in female livers, ☆= HCB in ovaries.

20% (fresh weight) in December to 6% in May. Fig. 2 illustrates, in accordance with these findings, the seasonal cycle of lipids in female dab livers. The neutral lipid fraction (storage lipids) decreases continuously parallel to the total lipid content, reaching its minimum in April, whereas the polar lipid fraction appears to be constant.

At the beginning of the spawning period in February (Htun-Han 1978a), Σ-PCB and HCB contamination of livers exhibited their lowest levels (Fig. 3); however, Σ-PCB and HCB in ovaries increased. Maximal Σ-PCB and HCB burden in ovaries coincides with a minimum of neutral lipids in female livers.

These findings may be explained by a transfer of organochlorines from livers to ovaries during periods of unfavourable food conditions.

This investigation is part of the project "Zirkulation und Schadstoffumsatz in der Nordsee (ZISCH), MFU 05765", supported by the German "Bundesminister für Forschung und Technologie". We thank the "Institut für Hydrobiologie und Fischereiwissenschaft" for collecting the dabs.

REFERENCES

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

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917

Boon JP (1984) Uptake, distribution and elimination of selected PCB components of Clophen A40 in juvenile sole (*Solea solea*) and effects of growth. In: Gray JS, Christiansen ME (ed) Marine Biology of Polar Regions and Effects of Stress on Marine Organisms, John Wiley & Sons Ltd., p 493-512

Boon JP, Duinker JC (1985) Kinetics of polychlorinated biphenyl (PCB) components in juvenile sole (*Solea solea*) in relation to concentrations in water and to lipid metabolism under conditions of starvation. Aquat Toxicol 7:119-134

Büther H (1988) Distribution of chlorinated organic compounds in livers of dab (*Limanda limanda*) of the southern and central North Sea. Mitt Geol-Paläont Inst Univ Hamburg 65:497-541

Bruggeman WA, Martron LBJM, Kooiman D, Hutzinger O (1981) Accumulation and elimination kinetics of di-, tri- and tetrachlorobiphenyls by goldfish after dietary and aqueous exposure. Chemosphere 10:811-832

Bruggeman WA, Opperhuizen A, Wijbenga A, Hutzinger O (1984) Bioaccumulation of super-lipophilic chemicals in fish. Toxicol Environ Chem 7:173-189

Duinker JC, Hillebrand MTJ (1983) Composition of PCB mixtures in biotic and abiotic marine compartiments. Bull Environ Contam Toxicol 31:25-32

Htun-Han M (1978a) The reproductive biology of the dab (*Limanda limanda (L.)*) in the North Sea: Seasonal changes in the ovary. J Fish Biol 13:351-359

Htun-Han M (1978b) The reproductive biology of the dab (*Limanda limanda (L.)*) in the North Sea: gonosomatic index, hepatosomatic index and condition factor. J Fish Biol 13:369-378

Knickmeyer R, Steinhart H (1989) On the distribution of polychlorinated biphenyl congeners and hexachlorobenzene in different tissues of dab (*Limanda limanda*) from the North Sea. Chemosphere 19:1309-1320

Schneider R (1980) Polychlorierte Biphenyle im Dorsch. Ph D Thesis Univ Kiel, FRG pp 22

Solbakken JE, Ingebrigtsen K, Palmork KH (1984) Comparative study on the fate of the polychlorinated biphenyl 2,4,5,2',4',5'-hexachlorobiphenyl and the polycyclic aromatic hydrocarbon phenanthrene in flounder (*Platichtys flesus*). Mar biol 83:239-246

Thormann RV (1989) Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ Sci Technol 23:699-707

Received January 17, 1990; accepted May 30, 1990.