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Significance of equilibrium partitioning and lipid composition in PCB accumulation by fish

by

R. Schneider

Institut für Meereskunde
an der Universitaet Kiel
Düsternbrooker Weg 20
D - 2300 Kiel, FRG

Abstract

In order to study the mechanism of the distribution of PCBs in organisms, a simple method for a coarse separation of extractable lipids according to their relative solubilities for PCBs was developed. This method was applied during the analyses of three tissues of cod caught in the Western Baltic. On a wet weight, dry weight, and extractable lipid basis, pollutant concentrations in the fillets, gonads, and livers differed significantly, whereas there was no significant difference between the tissues if their PCB concentrations were based on the non-polar fraction of their lipids.
This result may be regarded as a further proof that equilibrium partitioning between the surrounding water and certain body lipids is the primary mechanism of bioaccumulation of persistent lipophilic pollutants. Some important implications of this concept for aquatic environmental pollution studies are discussed.

Introduction

It is generally accepted that lipophilic pollutants, such as the organochlorine insecticides and PCBs, accumulate in those animal tissues with a high concentration of lipids. Thus, adiposity of a tissue or of an organism as a whole is generally assumed to be

a major factor influencing bioaccumulation of these pollutants, especially in gill-breathing aquatic animals. Nevertheless, adiposity has often been neglected in organochlorine residue analyses, and residue concentrations were only based on the wet or dry weights of the organisms or tissues analysed. It seems to be caused by this neglection, mainly, and by the circumstance that adiposities of the organisms often increase with trophic levels, that residue concentrations have often been found to be bioamplified up the food chains (e.g. WOODWELL, 1967; PORTMAN, 1967; METCALF et al., 1971; REINERT et al., 1972).

Other investigators did not find a corresponding increase of organochlorine levels when they analysed food chains from plankton to fish. Thus, they concluded "that food chain magnification of pollutants is not a useful concept with water-breathing animals" (HARVEY, 1974). HAMELINK et al. (1971) therefore proposed that exchange equilibria control the degree organochlorines are biomagnified in aquatic organisms. This concept was strongly supported by the experimental results of SCURA & THEILACKER (1977), who concluded that gill-breathing aquatic animals "will establish an equilibrium with the surrounding water which will depend on the concentration of the compound in the water and the solubility of the compound in the water and the lipid of the organism".

When we analysed a number of cod tissue samples and several of the most important cod prey organisms from one sampling site in the Western Baltic, we found our analytical results to be in line with this concept (SCHNEIDER, 1978) - except for one virtual inconsistence: The concept implies that almost all organochlorine residues occur in the lipid fraction, i.e. residue concentrations based on lipid weight should be equal at least for all tissues of an individual specimen, which obviously was not the case.

Taking into account that equilibria of partitioning are solubility-dependent and that "the extractable lipids" are a group of very dissimilar compounds, we then tried to analyse the relative solubilities for PCBs of the most important lipid classes. Our pre-tests (SCHNEIDER, 1978) indicated that, of the lipid

classes included, only the polar ones, namely phospho-, glyco-, and sphingolipids, are unable to dissolve appreciable amounts of PCBs. The aim of this present research was, thus, to establish whether PCB concentrations based on the weights of the relatively non-polar lipid fraction are equal for several tissues of the same cod.

Material and methods

In april 1978, each five spawning male and female cod, and in may 1979, two spawning females were caught by standard trawl near the centre of Kiel Bay. They were wrapped in aluminium foil, placed in polyethylene bags and deep-frozen (-20° C) aboard. In the laboratory, length, weight, age, sex, liver and gonad weights of each fish were determined immediately after thawing. Livers, gonads, and fillets were dissected, individually packed, and re-frozen until analysis.

In order to determine their water contents, all tissue samples were freeze-dried prior to analysis. After pulverization of the samples, aliquots were extracted in a Soxhlet-apparatus with a mixture of 10% acetone in n-hexane (nanograde) for 8 hours. The extracts were evaporated to dryness in tared flasks in order to determine the extractable lipid contents. The standard deviation of these determinations was below 1%.

The lipid remainder was divided into two proportions. One of them served for the determination of PCB content, the other one for the isolation of the non-polar lipid fraction. For the latter purpose, the following technique was modified from the methods described by CARROLL (1961, 1963): A chromatographic column (16 mm I.D.) was filled with 4g of dry Florisil (60-100 mesh). The proportion of the lipid extract was dissolved in 1 ml of n-hexane; this solution was transferred onto the column. The relatively non-polar lipid fraction, consisting of glycerides, free fatty acids, cholesterol and its esters mainly, were then eluted into a tared flask with 50 ml of a mixture of 25% hexane in acetone, whereas the polar fraction was retained by the column,

as confirmed by thin layer chromatography on silica. The eluate was evaporated to dryness and weighed. The mass of eluted materials was calculated as the percentage of the initial lipid mass to give the "fat" content of the extractable lipids.

It has to be noted that "fat" will denote this fraction in the following. However wrong this nomenclature may be biochemically, according to the similarities in some relevant properties of the lipids comprised, this denotation seems to be preferable to the common use of residue analysts of putting the terms "fat" and "lipid" synonymously.

The reproducibility of this technique is about ± 1.4%, as calculated from the results of parallel analyses of an ovarian lipid extract. Under the conditions described above, the Florisil column retained up to 150 mg of polar lipids; thus, a maximum of 150 mg of total extractable lipids were used for "fat" determinations.

The method applied for clean-up of extracts and separation of PCBs from pesticides was described earlier (SCHNEIDER & OSTERROHT, 1977). The gas chromatograph employed for the estimation of PCBs was a HP 5730A equipped with a pulsed ^{63}Ni-detector and coupled to a HP 3380A integrator. The instrument was fitted with a 2m x 2mm I.D. silanized glass column packed with 4% OV 101/6% QF-1 on 80-100 mesh Chromosorb W AW DMCS, which was run isothermally at 220°C with 30 ml/min argon/methane.

PCBs were quantified by comparison of the areas of the three major peaks of Clophen A60 in the gas chromatograms. The standard deviation of three parallel analyses of the 1978/79 ICES inter-calibration sample was 2.2%.

Results and discussion

To allow for comparison of the basic parameters, the tissues' PCB contents were calculated as concentrations per unit weight of wet tissue, dry tissue, extractable lipids, and "fat". The arithmetical means of these data are given in table 1 and in figure 1:

Table 1: Mean PCB concentrations (± standard deviation) of the cod tissues analysed (n = 12 each)

conc. basis	fillets	gonads	livers
ng/g wet weight	9 (4)	40 (27)	2240 (1150)
ng/g dry weight	51 (23)	319 (219)	4490 (1770)
µg/g extr. lipid	2.62 (1.20)	2.85 (1.50)	6.47 (2.05)
µg/g "fat"	7.92 (3.24)	7.82 (3.35)	7.69 (2.57)

Obviously, the mean PCB concentrations in the tissues differed significantly if based on wet weight, dry weight, and extractable lipids, whereas there was no significant difference between them on a "fat" basis. Significances of the differences were tested by analyses of variance.

Since the cod differed both in length (30-48 cm) and e.g. in liver-somatic index (1.6 - 6.8) it was thought necessary to compare not only the means of the absolute concentrations but also the distribution patterns of PCBs in the tissues of each fish. Therefore, according to MITCHELL et al. (1977), the relative PCB concentrations of the tissues (defined as PCB concentration in fillet or gonad of a specimen x 100 / PCB concentration in liver of the same specimen) were calculated. The means of these data are given in table 2 and figure 2.

Table 2: Mean relative PCB concentrations (± standard deviation) of the cod tissues analysed (Relative concentrations were calculated for each fish on each basis before mean values were taken)

basis	fillets	gonads	(livers)
wet weight	0.48 (0.35)	2.13 (2.18)	100
dry weight	1.23 (0.51)	7.98 (5.32)	100
extractable lipids	39.4 (8.8)	43.5 (10.7)	100
"fat"	103.5 (20.3)	103.3 (15.8)	100

The results indicate that the concentrations of lipophilic pollutants, at least of PCBs, in the tissues of an animal are, to the largest extent, proportional to the concentrations of "fat". Physico-chemically, this direct proportionality to the

group of extractable lipids such comprised seems possible for two reasons:

- It has been indicated that both groups of lipids ("fat" and polar lipids) differ widely in their solubilities for PCBs (SCHNEIDER, 1978).
- On a cellular level, both groups are known to occur in different compartments, the polar lipids being the main structural elements of the membrane systems, whereas "fats" are mainly present in discrete "fat globules" (which, by the way, are known to be the stores of lipophilic vitamins). Thus, both groups need not influence each other's solubility properties.

The distribution pattern of PCBs amongst the tissues analysed suggests that any PCBs absorbed by the fish (accountless whether from their food or from the surrounding water) are distributed amongst the "fat" in the tissues. Since it must be assumed from these results that the PCBs in the tissues are present in true solution, the process is likely to be a physical rather than a biochemical one. "Presumably the serum transports the pollutant, and tissues reach a dynamic equilibrium with the serum and hence with each other", as stated for DDT by MITCHELL et al. (1977). The uniformity of the "fat" based concentrations further suggests that there is no need of explaining the relatively low concentrations of DDT in trout brains, which HOLDEN (1962) found to be in contrast to their high contents of extractable lipids, by the blood-brain barrier being relatively impermeable to DDT: brain lipids are very well known to contain glyco- and sphingo-lipids, mainly, and to be poor in "fat".

Such, provided that PCBs are distributed amongst the tissues without being hindered by any physiological barrier, that they are present in the "fat" of the tissues in true solution, and that the serum is in equilibrium with the tissues - why shouldn't the serum on its way through the gills establish an equilibrium with the surrounding water, too?

The basic mechanism of exchange between tissues and serum is only imaginable as diffusion. The physicochemical way to describe an equilibrium of a diffusing solute between two immiscible phases

("fat" and water) is given by Nernst's equation, which may be formulated in this context:

$$\frac{\text{PCB concentration in "fat"}}{\text{PCB concentration in water}} = \text{constant}$$

Identical biochemical composition of fish and identical experimental conditions provided, any variation in the aqueous PCB concentration must necessarily result in the same bioaccumulation factor, then:

$$\frac{\text{PCB concentrn in fish}}{\text{PCB concentrn in water}} = \text{bioaccumul. factor} = \text{constant}$$

In fact, constant factors of this type were found e.g. by HANSEN (1976) for lindane in Gasterosteus aculeatus, by MATSUMURA (1976) for DDT in Pimephales promelas, and by DE FOE et al. (1978) for two Aroclors in the same species!

Nernst's law also implies that, if the solute is in equilibrium with both phases, the ratio between its concentrations in the phases is equal to the ratio between its solubilities in them. Provided that several lipophilic pollutants are rather similar in their "fat" solubilities but differ markedly in their water solubilities, their bioaccumulation factors must therefore depend on their water solubilities: It has often been stated that the bioaccumulation factors of lindane (water solubility about 10 ppm), dieldrin (280 ppb), and DDT (1.2 ppb) generally increase in this order (e.g. ADDISON, 1976).

A similar proof for the validity of the concept was given by HAQUE et al. (1977), who found a significant correlation between the octanol/water partition coefficients and the trout muscle/water bioaccumulation factors of eight lipophilic compounds tested...

Thus, it may be concluded that the concept proposed by SCURA & THEILACKER (1977, see above) is doubtlessly a valuable approach to the basic mechanism involved in bioaccumulation of lipophilic pollutants in gill-breathing aquatic animals; it has, for the first time, been achieved in this study presented to identify the lipid fraction partitioning PCBs with the surrounding water. Apart from the theoretical progress presented, there are some important implications for future pollution studies:

Bioaccumulation factors of PCBs between aquatic organisms and water have often been calculated to be in the range of 10^4 - 10^5, as summarized by ERNST (1973). The concentration data in fish used for these calculations were based on wet weights. Regarding the basic mechanism of accumulation, however, it seems preferable to calculate the organism's PCB concentrations on a "fat" basis, bioaccumulation factors then being equal to partition coefficients. The advantage would be that they offer a means to determine both mean pollutant concentrations in the water integrated over space and time as well as subdetectable aqueous pollutant concentrations. To give a preliminary example: On a "fat" basis, mean PCB concentrations in the tissues analysed during this study were about 7.5 ppm; the most recent data on PCB concentrations in Western Baltic water were published by STADLER & ZIEBARTH (1976) with a mean value of about 3 pptr. Supposing that in the meantime concentrations have decreased rather, the accumulation factor between cod "fat" and seawater, i.e. the partition coefficient, is at least 2.5×10^6. SCHNEIDER & KOCK (in preparation) found krill and fish from the Bransfield Strait (Antarctica) to contain about 0.5 ppm PCBs on a "fat" basis. Neglecting the influences of temperature, salinity, and probable differences in the "fat" compositions, this would mean that PCB concentrations in the water of Bransfield Strait are about 0.2 pptr. at maximum. Admittedly, this value may deviate from the real concentration widely; but the detection limit for direct water analyses are calculated to be 0.5 pptr. (STADLER, 1977)...

In account of the significant rôle of the lipids in the bioaccumulation of organochlorines, it is urgently recommended that lipid determination procedures are standardized at least for monitoring programmes as soon as possible. To base PCB concentration data on tissue (or animal) "fat" contents would offer the advantage that parallel analyses of different tissues of the same specimens are no longer necessary, as has been indicated in this study.

Many investigators have, in the past, used emulsifiers or organic solvents to introduce low-water-solubility compounds into the experimental water, when testing toxicities or bioaccumulation

capacities. According to Nernst's law, however, any slight increase in the water solubility of a lipophilic pollutant must necessarily result in a decrease in its accumulation factor and thus in its toxicity. It probably happened by this mistake in their experimental design, that MACEK & KORN (1970) could conclude that the food chain was the major source of organochlorines for fish in their natural environment...

Acknowledgements

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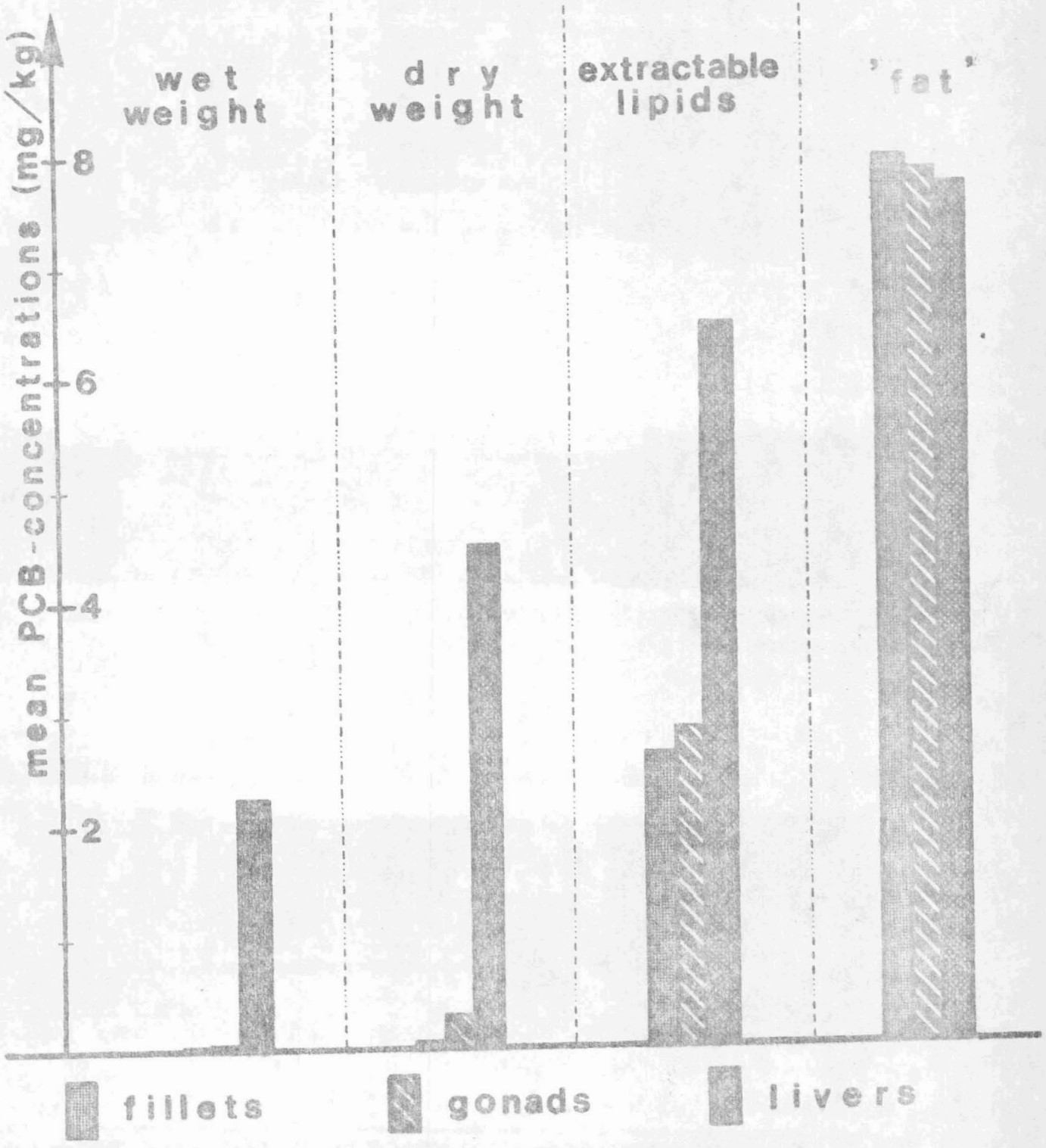

Figure 1: Mean PCB concentrations in the cod tissues analysed, calculated on four different bases

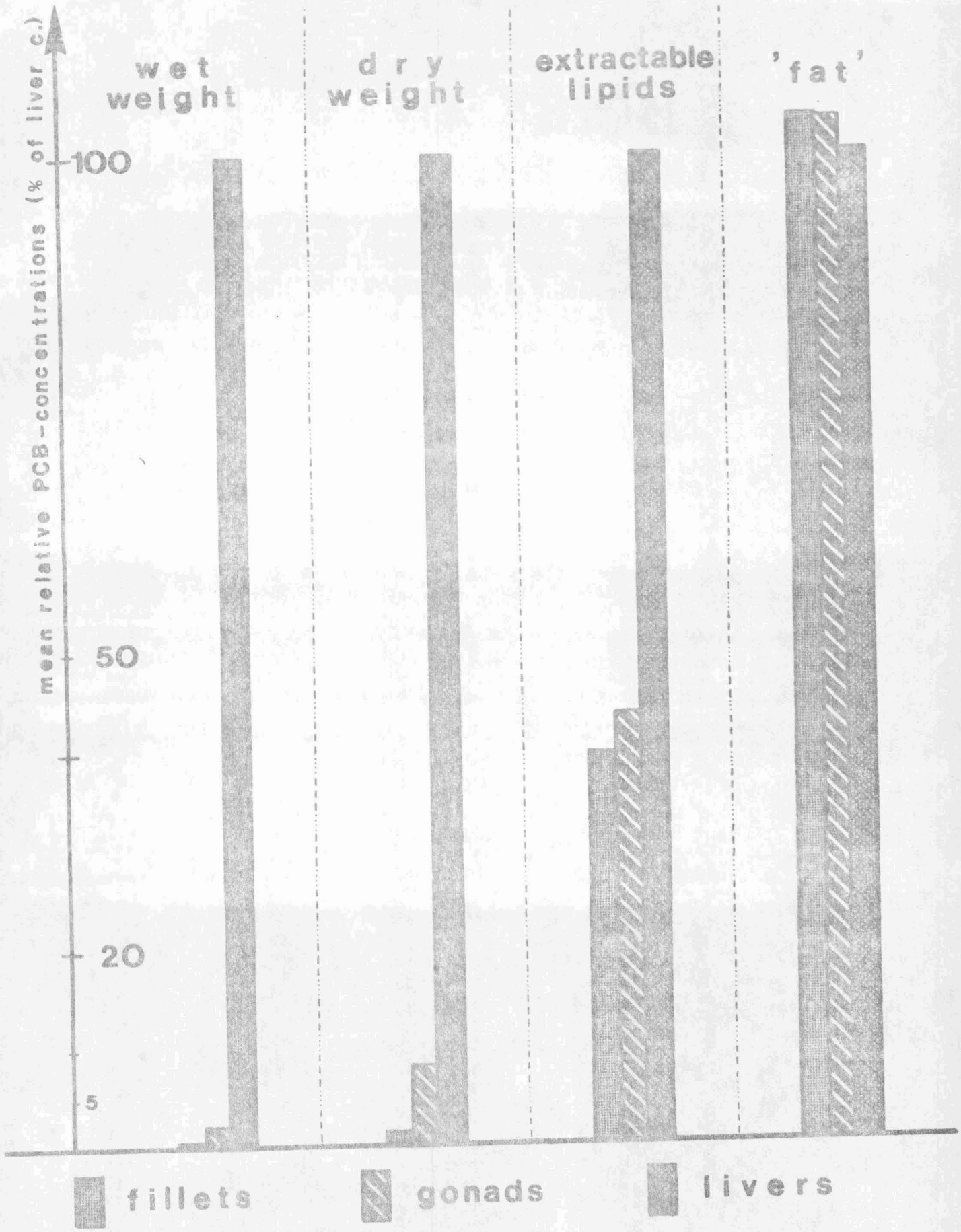

Figure 2: Mean relative PCB concentrations in the analysed tissues (concentration in tissue x 100/ concentration in liver of the same specimen)