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Fish physiology

ABSORPTION OF POLYCHLORINATED BIPHENYLS (PCB) THROUGH GILLS AND SKIN OF COMMON CARP, *CYPRINUS CARPIO* L.

WCHŁANIANIE POLICHLOROWANYCH BIFENYLI (PCB) PRZEZ SKÓRĘ I SKRZELA KARPIA *CYPRINUS CARPIO* L.

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Gills and skin of mirror carp were exposed separately to Clophen A-50 in the concentration of 200 μg/dm^3. After one hour, the content of polychlorinated biphenyls (PCB) was determined in the wet weight of the blood, muscles, and the skin as well as in the lipids of these tissues. It turned out that 63% of the PCB were absorbed through the gills, while only 32%—through the skin.

INTRODUCTION

Polychlorinated biphenyls (PCB) are compounds of two benzene rings in which chlorine takes place of one to ten atoms in a molecule. They are among the most durable synthetic compounds, resistant to pH changes and to prolonged heating. They do not conduct electrical current and exhibit low vapor pressure, high durability and toxicity (Fishbein 1972). Wide usage in many branches of industry (dielectric fluids, isolation materials, components of plastics, paints and packaging materials etc.) and low pace of biodegradation, caused that polychlorinated biphenyls constitute a persistent component of the environmental pollution. In bodies of water these compounds are poorly soluble in the water (0.04–0.20 mg/dm^3). Lipophilic PCB quickly enter the trophic cycle and their concentrations in fishes can be very high (Roots 1984, 1990; Roots and Sare 1987; Roots et al. 1988). Individual organs and tissues, however, show different bioaccumulation coefficients (Gooch and Hamdy 1983; Solbakken et al. 1984). Significantly higher concentrations of PCB are de-

tected in the visceral fat deposits, in the skin, liver, brain, gills, and gonads (Solbakken et al. 1984; Malius et al. 1986; Weigelt 1986).

Polychlorinated biphenyls alike others toxic substances enter fish organism with food. In a direct way they are absorbed from the water through the gills and skin.

In toxicological studies the gills and skin, despite fundamental anatomo-physiological differences (like surface area, sensitivity, mechanisms of selective permeability etc.) are usually treated jointly and referred to as the "body surface".

The present study was aimed at separate assessment of involvement of the gills and skin in the process of PCB absorption from the water. The Winterstein method (cited after Puczkow 1962) was used. The method was modified to enable selective contact of the water with either the gills or the fish skin.

The assessment was based on the quantitative analysis of PCB compounds accumulated in the blood, muscles, liver, and the fish skin.

MATERIAL AND METHODS

The study was conducted in spring and it was based on 26 mirror carp, weighing 320–470 g and originating in a cage culture. The experiment was performed in aerated tap water at 22 ±10°C (pH 7.8–8.2; oxygen content 8.2–9.4 mg/dm^3). The source of PCB was a product, commercially known as Clophen A-50, which was added to the water in the concentration of 200 μg/dm^3. The fish for the experiment were selected in random. The exposure time was 1 hour.

A special research setup (Fig. 1) was used to obtain separate water access the gills or to the skin. The setup consisted of a glass 40-dm^3 fish tank and of two 25-dm^3 Mariott bottles. The bottles were supplying the water of a constant speed of flow. The glass tank was divided with glass walls into 3 chambers of the volume 10, 10, and 20 dm^3 respectively. All chambers had individual overflow openings. In the wall between chamber I and chamber II there was a rigid tube mounted on the side of chamber II. The tube had 8 mm in diameter. In the wall between chamber II and chamber III there was an opening 9 × 15 cm with an elastic rubber membrane attached to its margin and partly covering its lumen. The setup was equipped with custom made mouth-pieces adjusted to size the mouth openings of the fish studied.

Procedure

The mouth-piece was inserted into the mouth opening of fish and fastened with an elastic band. The fish was placed in the tank and the mouth-piece was attached with the tube in chamber II. Due to such arrangement the fish was taking the water for respiration from chamber I. Its head was in chamber II, while the rest of the body—in chamber III. The

membrane tightly holding the fish immediately behind the head, prevented mixing of the water between chambers II and III.

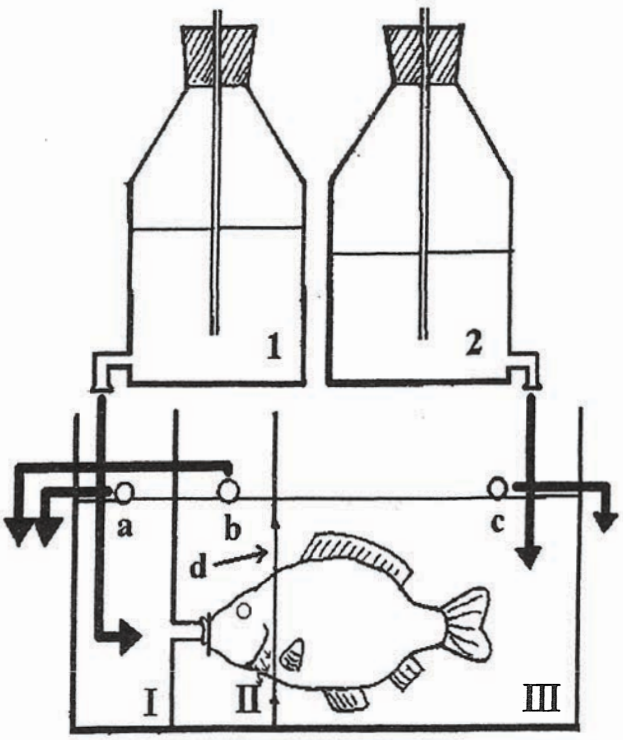

Fig. 1. Diagram of the water flow in the experimental setup
1, 2—Mariott bottles; I, II, III—glass tank chambers;
a, b, c—outflow holes; d—rubber membrane

The solution of Clophen A-50 was prepared in Mariott bottles.

When only the gills were exposed, Clophen was delivered from bottle No. 1, while bottle No. 2 supplied clean water. When the skin was exposed, the contaminated water was delivered form bottle No. 2, while clean water—from bottle No. 1. When the entire body surface was exposed, both Mariott bottles supplied concentration of Clophen.

The amounts of the water supplied, were adjusted to the efficiency of the respiratory movements of the gills of the fish studied. For the carp weighing 320–470 g these amounts were between 17 and 21 dm^3/h. The excessive water form the experimental aquarium flown out through the overflow openings. The openings were on the same level in all three chambers to maintain even hydrostatic pressure.

Analytic methods

The analytic samples consisted of 10-g portions of the muscle tissue and the liver, 2-g—of the skin, and 3-g—of the blood. The PCB content was determined using the method of Jansen et al. (1993). The lipids along with the PCB were extracted with acetone-hexane mixture (2.5 : 1), which was followed by extraction with hexane-ethyl-ether mixture (9 : 1). After evaporating down to the volume of 1.5 cm^3 the extracts were purified with 7-% SO_3 in concentrated H_2SO_4 and 5-% KOH in 96-% C_2H_5OH. The extract treated in such way was subjected to chromatographic separation in a gas-chromatograph GCHF 18.3 using a tritium ECD detector in the following conditions:

- glass column (4.0-m long 3.0 mm in diameter)
- column filling 2.8 GF1: 0.6% SF on Chromatosorb in 100–120 mash,
- temperature of the column and detector—200°C,
  carrier gas—nitrogen; flow—30 cm^3/min.

Quantitative identification of the compounds studied consisted in comparing the retention times of the sample peaks with the peaks of the standard (Clophen A-50). Qualitative determination was based on comparing heights of the peaks. The results were processed statistically using Student t-test.

RESULTS AND DISCUSSION

The results of the analytic study, shown in Tab. 1 indicate that the PCB content in the control samples ranged from 0.014 mg/kg of the wet mass of the blood to 0.038 mg/kg of the wet mass of the skin. After the intoxication, all of the organs and tissues studied, exhibited increase of the PCB content. The increase was dependent on the surface exposed.

When the water contaminated with Clophen A-50 had contact only with the gills, the average level of this PCB compound determined for the wet mass was 0.589 in the muscles, 0.214—in the liver, and 0.087 mg/kg—in the skin. In relation to the lipids, the highest concentration was observed in the blood (99.442 mg/kg), while the lowest—in the skin (0.506 mg/kg).

In the case of the skin exposure to Clophen A-50 the levels of PCB were significantly lower in the wet mass of the blood (0.092), muscles (0.051), and the liver (0.037 mg/kg). They were higher, however, in the skin—0.170 mg/kg (of the wet mass). In the lipids the PCB concentrations ranged from 0.571 in the liver to 18.699 mg/kg in the blood.

After 1-hour exposure of the whole fish the PCB levels were as follows: 0.793 in the blood, 0.194 in the muscles, 0.261 in the liver, and 0.226 mg/kg in the skin. In the lipids the values were: 116.022; 5.517; 4.269, and 1.249 mg/kg respectively (Tab. 1).

The outcome of the present study is consistent with the results of the other authors who stated the water-born polychlorinated biphenyls, as lipophilic compounds can easy cross cellular and subcellular membranes, entering fish organism and they are characterized by high bioaccumulation coefficients (Tutsakawa 1973; Cundel 1974; Harvey et al. 1974; Lichtenstein et al. 1974; Dexter and Field 1989).

The acquired results of the quantitative analyses were used for preliminary assessment of the role of the gills and skin in the process of direct absorption of PCB from the water. The amount of PCB entering the organism when entire fish (gills and skin) is exposed was assumed as 100-% intake through the body surface. Comparison of the results based on this assumption revealed (Fig. 2) that the fish with only gills exposed to Clophen A-50 absorbed, in average, 63% of the PCB compounds. On the other hand the fish with only their shin exposed, absorbed some 32% of the PCB.

The gills of fishes serve different functions. In addition to the exchange of the respiratory gases, the gills are also responsible for ionic exchange in the process of osmoregulation, and in the process of maintaining acid-base balance. The gills also excrete nitrogen compounds. The gills are particularly sensitive for lowering pH (Mc Donald 1983), for heavy metal content in the water (Verbost et al. 1987), detergent content (Abel et al. 1975), pesticides (Eller 1971; Jara 1973; Virtanen 1986). The histopathologic changes (hyperemia, epithelial erosion, necrosis) occur, however, only when the fishes are exposed to lethal concentrations of the toxicants (Mallatt 1985).

The present study, where 200 $\mu g/dm^3$ concentration of Clophen A-50 was used for 1 hour, no visible changes of the gill picture were observed in the experimental fish.

The most characteristic non-specific response of the fish skin to the adverse factors (mechanical traumas, thermal shock, toxic compounds) is intensified production of mucus (Mitall 1974; Dayae and Garside 1976; Pickering et al. 1982).

The above symptom has not been observed in the present study, which would indicate that the concentrations of Clophen A-50 used was too low to trigger reflexive defense reaction of the skin. There has been a common perception that fish skin is little permeable for substances dissolved in the water, especially if covered with the scales (Jara 1973).

Table 1

PCB intake through the gills and skin of carp. Water concentration of Clophen A-50—200 $\mu g \cdot dm^{-3}$; exposure time—1 hour ($\bar{x}$ ±SD)

Exposed surface	Number of fish	PCB content in the wet mass [$mg \cdot kg^{-1}$]				Lipid content [%]				PCB content in the lipids [$mg \cdot kg^{-1}$]			
		Blood	Muscles	Liver	Skin	Blood	Muscles	Liver	Skin	Blood	Muscles	Liver	Skin
Gills	6	0.589 ±0.035	0.113 ±0.010	0.214 ±0.014	0.087 ±0.008	0.59 ±0.02	3.56 ±0.31	6.52 ±0.31	17.52 ±1.03	99.442 ±2.738	3.392 ±0.319	3.392 ±0.174	0.506 ±0.028
Skin	6	0.092 ±0.023	0.051 ±0.003	0.037 ±0.005	0.170 ±0.030	0.52 ±0.07	3.36 ±0.29	6.43 ±0.32	17.67 ±1.12	18.699 ±3.367	1.458 ±0.219	0.571 ±0.054	0.827 ±0.143
Gills and skin	6	0.793 ±0.066	0.194 ±0.015	0.261 ±0.010	0.226 ±0.026	0.54 ±0.06	3.38 ±0.27	6.64 ±0.42	17.19 ±1.22	116.022 ±13.983	5.517 ±0.672	4.269 ±0.046	1.249 ±0.103
Control	8	0.014 ±0.006	0.021 ±0.006	0.019 ±0.008	0.038 ±0.011	0.56 ±0.04	3.43 ±0.31	6.48 ±0.39	17.34 ±1.27	2.500 ±0.257	0.088 ±0.014	0.274 ±0.061	0.189 ±0.062

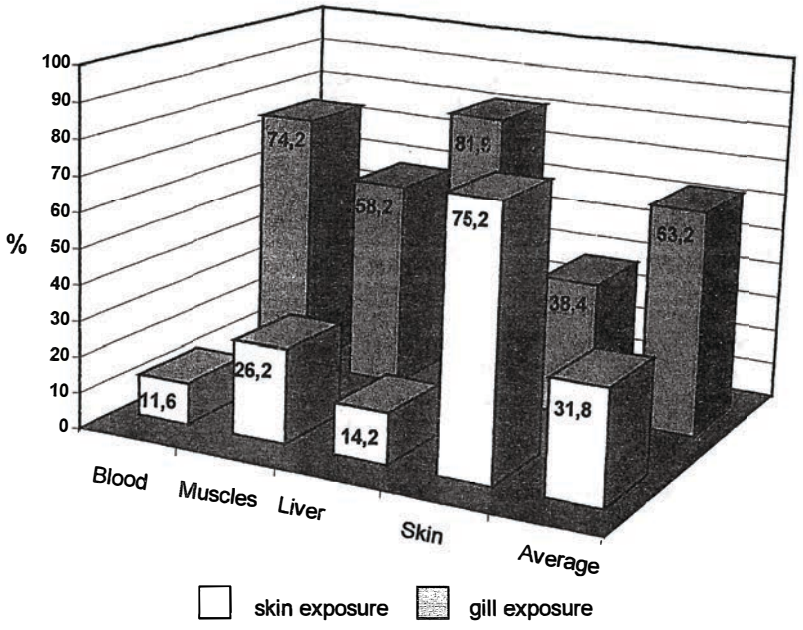

Fig. 2. Proportional role of the gills and skin in PCB intake from the water to the carp tissues following 1-hour exposure to Clophen A-50. Exposure of the entire body surface—100%; Concentration of Clophen A-50 in the water—200 μg/dm^3

The present results showed that for the mirror carp, having very few scales, the skin surface plays a significant role in direct absorption of the PCB compounds. The latter accumulate chiefly in the skin lipids (Tab. 1) from where, through the blood, they penetrate the muscles and the liver.

CONCLUSIONS

1. One-hour exposure of carp for 200 μg/dm^3 concentration of Clophen A-50 caused statistically significant increase of PCB content in the wet mass and lipids of the blood, muscles, liver, and skin.
2. The highest content of the PCB was observed in the fish, where both skin and gills were exposed. These values were assumed as 100% absorption. The fish with only their gills exposed absorbed some 63% of the PCB. The fish with only their skin exposed absorbed only some 32% of the PCB.

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WCHŁANIANIE POLICHLOROWANYCH BIFENYLI PRZEZ SKRZELA I SKÓRĘ KARPIA (*CYPRINUS CARPIO* L.)

STRESZCZENIE

Badano wchłanianie związków PCB z wody przez skrzela i skórę ryb. Doswiadczenie wykonano w warunkach akwaryjnych na 26 karpiach lustrzeniach o masie jednostkowej 320–470 g, które przez okres 1 godziny przetrzymywano w koncentracji 200 μg/dm^3 preparatu Clophen A-50.

Ekspozycji poddano całą powierzchnię ciała (skóra i skrzela) oraz tylko skrzela i tylko skórę. Następnie u badanego materiału określono zawartość PCB w mokrej masie krwi, wątroby, mięśni i skóry oraz w lipidach tych tkanek.

Najwyższą zawartość PCB stwierdzono w tkankach ryb, u których ekspozycji na Clophen A-50 poddano całą powierzchnię ciała (wartości przyjęte jako 100% wchłaniania przez powierzchnię ciała), niższą przy ekspozycji tylko skrzeli – ok. 63% i najniższą przy ekspozycji tylko skóry – ok. 32%.

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