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CONCENTRATION OF POLYCHLORINATED BIPHENYLS IN DIFFERENT TISSUES OF CULTURED RAINBOW TROUT, (Salmo gairdneri).

by

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ABSTRACT

Trunk muscle, ventral muscle, visceral fat and liver from cultured rainbow trout (Salmo gairdneri) were analysed for PCB congeners. The congeners quantified were components 52, 49, 44, 101, 118, 138, 187, 183, 128, 180, 170, and 194. The concentrations of the individual PCB congeners and the sum of the quantified congeners (sum PCB) are given.

No statistical differences were found between the sum PCB concentrations on a lipid basis in the trunk muscle, ventral muscle and visceral fat. The concentrations found ranged from 0.24 0.45 mg/kg. The concentrations measured in the liver were significantly lower than the concentrations in the other tissues, with concentrations from 0.16 to 0.29 mg/kg. On a wet weight basis there were no significant differences between the concentrations in the ventral muscle and the visceral fat which had values from 0.17 to 0.35 mg/kg. In the trunk muscle values from 0.01 to 0.03 mg/kg were found and in the liver values from 0.01 to 0.02mg/kg. The concentrations of the individual PCB congeners and the sum PCB concentrations were low in all the tissues analysed

INTRODUCTION

Polychlorinated biphenyls (PCB´s) were first discovered in environmental samples in Sweden (Jensen, 1966). They are strongly lipophilic compounds with a high octanol-water partition coefficient and have a high bioaccumulation factor (Rapaport and Eisenreich, 1984, Chiou, 1985, Oliver and Niimi, 1985). Mammals and birds have been found to metabolize PCB´s (Hutzinger et al., 1972, Bergman, 1983), whereas, fish have shown a very low degree of metabolism (Melancon and Lech, 1976, Hinz and Matsumura, 1977, McKim and Heath, 1983). Laboratory experiments have shown that fish accumulate PCB´s from water (Zitko and Carson, 1977, Broyles and Noveck, 1979, McKim and Heath, 1983) and via the diet (Lieb, Bills and Sinnhuber, 1974; Solbakken, Ingebriktsen and Palmork, 1984, Boon, Oudejans and Duinker, 1984). In lake trout the diet was found to be the most important source for PCB contamination (Thomann and Connolly, 1984).

Packed columns have been used for elution of PCB components in gas chromatography. The resolution of the PCB components were poor and the chromatograms contained only a few peaks, each peak consisting of a number of PCB congeners. The availability of capillary columns in the late 1970´s improved the analytical techniques and separation and identification of individual components is now possible. However, complete resolution of all the individual congeners using one column is still not feasible with the present techniques. Nevertheless, almost 60 of the 209 different possible congeners have been identified in marine biological samples. Studies have been performed on both marine invertebrates and marine fish (Zell, Neu and Ballschmiter, 1978, Taush, Stehlik and Wihlidal, 1981, Duinker and Hillebrand, 1983, Tuinstra, et al., 1983, Duinker, Hillebrand and Boon, 1983, Boon et al., 1985).

A major problem in PCB analyses is co-elution of PCB congeners. It is necessary to identify all the PCB congeners present in the sample to obtain an estimate of the total PCB concentration. Complete separation of most of the 209 congeners is not possible unless more columns of different polarity is used. This is yet not feasible in routine analyses. Research done by Duinker et al. (1987) has led to the following basic conditions to be fullfilled before any selection of congeners should take place:

1. The congeners should appear as single peaks on a SE-54 capillary column.

2. The congeners should be relevant for the marine environment.

3. The congeners should be available in sufficiently pure form for quantitative and qualitative purposes.

A standard solution has been made up by Duinker and his group in Kiel, consisting of the following IUPAC numbers: 18, 26, 52, 49, 44, 101, 151, 149, 118, 138, 187, 183, 128, 180, 170, and 194.(Duinker et al, 1987).

Analyses of PCB levels in commercially important fish species in the North Sea, has been organized by ICES (Anon 1974, 1977a, b, and c, 1980 a and b, and 1984). Monitoring programs of the PCB levels in cultured fish are not conducted in the same manner and few studies have so fare been published on the PCB levels in cultured salmonid fish. The purpose of this study was to get an idea of the concentration levels in cultured salmonid fish and to prepare a convenient method for quality control of cultured fish.

MATERIALS AND METHODS

The analyses were performed on 3 year old rainbow trout (*Salmo gairdneri*) obtained from the Institute of Marine Research, Matre Aquaculture Station (Matredal, Norway). The station is located in Masfjorden, a non-industrial, sparsely populated area on the west coast of Norway. The fish had been grown in floating net cages in the sea for 1.5 year and fed commercially available dry pellets (Tess Fish Feed, ELITE PLUSS,Skretting, Stavanger, Norway).

A total of 10 fish and 4 different types of tissue from each fish were analysed. Muscle samples, 25-40 g, were taken from the trunk muscle between the dorsal fin and the lateral line. Samples of liver (20-30 g), visceral fat (15-20 g) and ventral muscle (5-10 g) were taken following the same procedure. The samples were frozen at -20 °C.

Sample preparation

Thawed samples were ground in a high speed blender with anhydrous sodium sulphate and hexane. The fat content of the samples was determined by gravimetry. Clean up was performed by heating the samples for 2 hours with 2N KOH. The PCB´s and DDE were then extracted with pentane.

Instrumentation

The samples were analysed on a Hewlett Packard 5710A gas chromatograph equipped with a ^{63}Ni electron capture detector. Identification and quantification were performed using a Hewlett Packard 5880A Series GC Terminal integrator. The chromatographic conditions were set as listed in Table I.

PCB determination and quantification

An internal standard was used for the quantification of the different PCB congeners and they were named according to the the IUPAC rules as described by Ballschmiter and Zell (1980). Previous analyses of the different tissues had shown an absence of the PCB congener IUPAC No. 53, which therefore was chosen as the internal standard. A standard solution containing a mixture of 75 different PCB congeners were used for identification. The PCB peaks in the samples were identified by

Table I. Condition for capillary column gas chromatography of the PCB congeners.

Gas chromatography	:Hewlett Packard 5710A
EC-detector	:^{63}Ni
Column	:50m x 0.33mm i.d. Se-54 fused silica
Carrier gas	:Hydrogen, linear velocity 35 cm/sec
Make-up gas	:Nitrogen, 30 ml/min
Septum purge	:5 ml/min
Injection temp.	:250°C
Detector temp.	:250°C
Temp. prog.	:100°C initial 4°C /min to final temp. 260°C
Injection	:2 microl splitless

retention times and the concentration of 12 of the congeners calculated using the response factors. The quantified congeners were chosen according to Duinker et. al. (1987), who used the following criteria for the selection of the congeners:

1. The congeners should appear as single peaks on a SE-54 capillary column.

2. The congeners should be relevant for the marine environment.

3. The congeners should be available in sufficiently pure form for quantitative and qualitative purposes.

The congeners that they included in their standard solution consisted of the following IUPAC numbers: 18, 26, 52, 49, 44, 101, 151, 149, 118, 138, 187, 183, 128, 180, 170, and 194.(Duinker et al., 1987).

In our study the following congeners were included: 52, 49, 44, 101, 118, 138, 187, 183, 128, 180, 170, and 194. The congeners 18, 26, 151, 149 were not included in our standard mixture at the time of the analyses and could for that reason not be quantified.

The estimate of the sum PCB concentration was determined by adding up the amount of the different identified PCB congeners that we used.

A student t-test was used to look for differences in PCB concentrations between different tissues. The level of significans was set to $p<0.05$ for all tests.

RESULTS

The analyses were carried out on a total of 10 fish. The length and weight of the fish and the lipid content in the analysed tissues are given in table II. There were no significant differences in lipid content in the ventral muscle and the visceral fat, both consisting of approximately 70 % fat. The fat content in the liver and the muscle

were much lower, with the lowest values measured in the liver (Table II).

Table II. Length and weight of rainbow trout (*Salmo gairdneri*) and percentage of hexane extractable lipids in trunk muscle, ventral muscle, visceral fat and liver. Mean values and standard deviations are also given.

Fish	Length	Weight	% Lipid			
No.	cm	g	Trunk	Ventral	Visceral fat	Liver
1	47	2222	6.6	75.7	81.0	8.5
2	54	2856	6.0	70.7	75.0	4.7
3	55	2190	7.3	79.0	72.9	4.6
4	52	2127	7.4	69.2	76.1	6.1
5	55	2184	6,6	61.2	73.5	4.8
6	55	2180	3.9	59.5	72.7	3.6
7	52	2116	4.7	79.2	71.2	5.5
8	56	2206	9.0	70.4	73.5	4.4
9	56	2193	7.2	70.1	70.7	3.0
10	48	1531	4.3	69.4	74.0	4.9
Mean±Sd	53±3	2181±314	6.3±1.5	70.4±6.2	4.8±1.0	74.0±2.8

The sum PCB concentrations based on the lipid weight in the different tissues are given in table III. The levels found were low and the variation in concentration within the tissues was small. There were no statistical differences between the sum PCB concentration in the trunk muscle, ventral muscle and visceral fat. The values ranged from 0.24 to 0.45 mg/kg. The concentration measured in the liver was significantly lower than the concentrations in the other tissues and ranged from 0.16 to 0.29 mg/kg.

The corresponding values given on a wet weight basis are given in table IV. There were no significant differences between the concentration in the ventral muscle and the visceral fat. The values ranged from 0.17 to 0.35 mg/kg. Low values were found in the liver and trunk muscle. The values ranged from 0.01 to 0.03 mg/kg in the trunk muscle and from

Table III. Concentrations (mg/kg lipid weight) of sum PCB´s in trunk muscle,ventral muscle, visceral fat and liver of rainbow trout (*Salmo gairdneri*).

Fish No.	Trunk muscle	Ventral muscle	Visceral fat	Liver
1	0.28	0.29	0.27	0.24
2	0.36	0.25	0.22	0.23
3	0.32	0.45	0.42	0.21
4	0.24	0.30	0.30	0.19
5	0.29	0.31	0.32	0.29
6	0.36	0.30	0.33	n.a.
7	0.24	n.a.	0.34	0.21
8	0.34	0.35	0.33	0.16
9	0.31	0.28	0.32	0.23
10	0.31	0.26	0.31	0.20
Mean±sd	0.31 ± 0.04	0.31 ± 0.06	0.32 ± 0.05	0.22± 0.04

n.a. not analysed

Table IV. Concentrations (mg/kg wet weight) of sum PCB´s in trunk muscle, ventral muscle visceral fat and liver of rainbow trout *Salmo gairdneri*).

Fish No.	Trunk muscle	Ventral muscle	Visceral fat	Liver
1	0.02	0.22	0.22	0.02
2	0.01	0.18	0.17	0.01
3	0.02	0.35	0.30	0.01
4	0.02	0.17	0.22	0.01
5	0.02	0.19	0.24	0.01
6	0.01	0.18	0.28	n.a.
7	0.01	n.a.	0.24	0.01
8	0.03	0.25	0.21	0.01
9	0.02	0.19	0.23	0.01
10	0.01	0.18	0.23	0.01
Mean±sd	0.02± 0.01	0.21 ± 0.06	0.234 ± 0.04	0.01 ± 0.00

n.a. not analysed

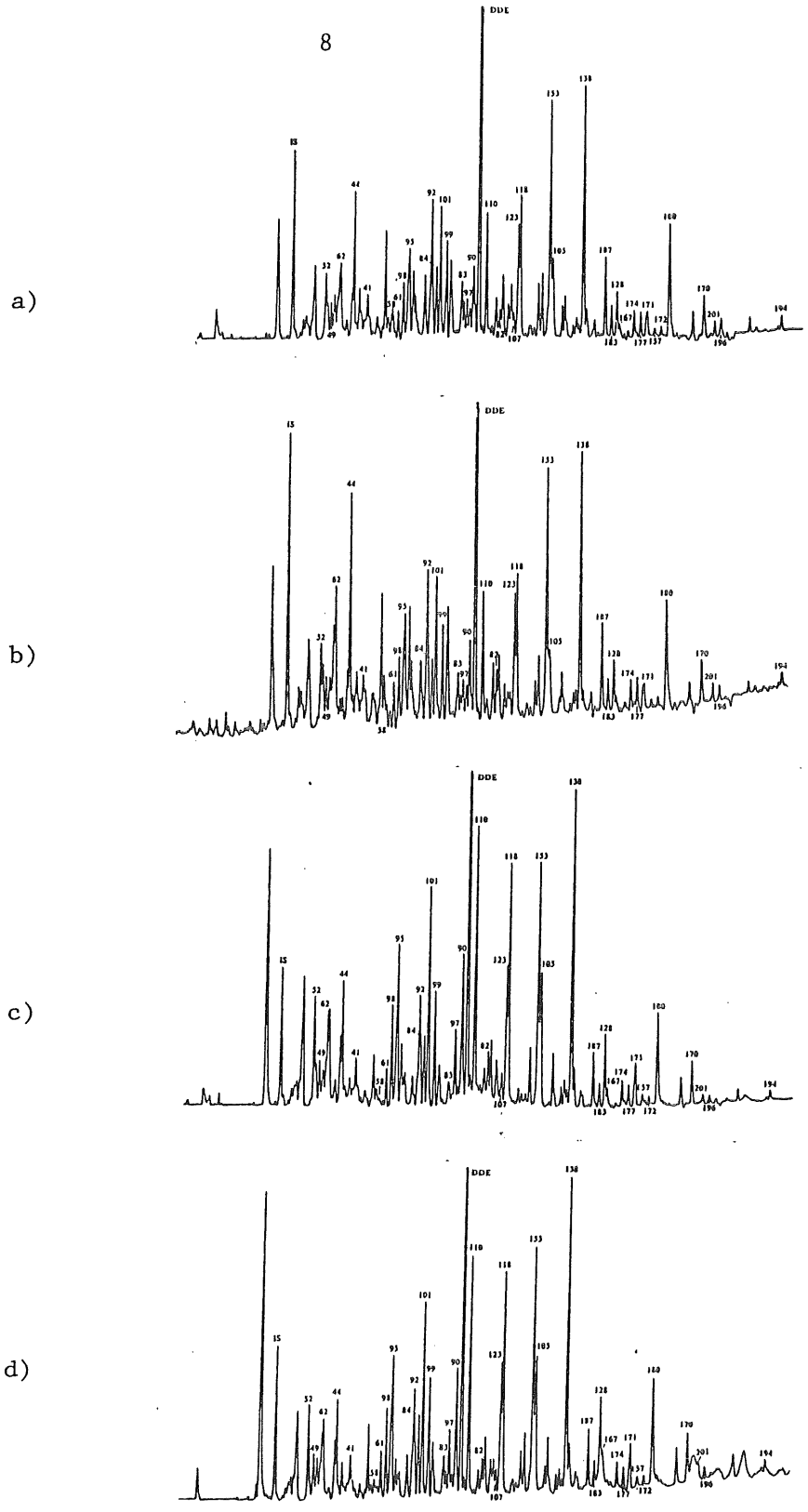

Figure 1. Capillary gas chromatograms of PCB congeners in trunk muscle (a), ventral muscle (b), visceral fat (c) and liver (d). The internal standard (component no. 53) have been labelled IS. Numbering according to the IUPAC rules as discribed by Ballschmiter and Zell (1980).

0.01 to 0.02 mg/kg in the liver. There were significant statistical differences in contamination levels between the liver and the trunk muscle.

Chromatograms of the PCB congeners in the different tissues are shown in figures 1 a, b, c and d. Table V shows the concentrations of the individual components in the different tissues based on the lipid weight. Of the quantified PCB congeners the tetrachlorobiphenyls, Nos.52 and 44, the pentachlorobiphenyls, Nos. 101 and 118, the hexachlorobiphenyl No.138 and the heptachlorobiphenyl No. 180 were the dominant congeners. Congener No. 138 were found in highest concentrations with values from 0.03 to 0.06 mg/kg fat.

Table V. Mean concentrations and standard deviation of individual PCB components in different tissues of rainbow trout (*Salmo gairdneri*) given as mg/kg hexane extractable lipids.

Comp. No.	Trunk muscle	Ventral muscle	Visceral fat	Liver
52	0.030 ± 0.005	0.029 ± 0.005	0.037 ± 0.007	0.026 ± 0.008
49	0.010 ± 0.002	0.010 ± 0.001	0.010 ± 0.001	0.008 ± 0.002
44	0.049 ± 0.008	0.038 ± 0.009	0.058 ± 0.007	0.038 ± 0.006
101	0.042 ± 0.005	0.050 ± 0.012	0.049 ± 0.011	0.028 ± 0.003
118	0.039 ± 0.005	0.050 ± 0.013	0.043 ± 0.011	0.025 ± 0.003
138	0.053 ± 0.010	0.060 ± 0.012	0.056 ± 0.010	0.034 ± 0.004
187	0.010 ± 0.002	0.008 ± 0.001	0.008 ± 0.001	0.006 ± 0.001
183	0.009 ± 0.001	0.007 ± 0.002	0.006 ± 0.001	0.004 ± 0.001
128	0.008.± 0.001	0.008 ± 0.003	0.008 ± 0.002	0.005 ± 0.001
180	0.039 ± 0.008	0.032 ± 0.005	0.032 ± 0.007	0.022 ± 0.003
170	0.012 ± 0.003	0.010 ± 0.002	0.010 ± 0.003	0.007 ± 0.001
194	0.005 ± 0.002	0.003 ± 0.001	0.003 ± 0.001	0.002 ± 0.002

Table VI show the concentrations of the individual components in the different tissues based on wet weight.The concentration in the liver and the trunk muscle are all < 0.005 mg/kg whereas the values in the visceral fat and the ventral muscle where much higher. The percentage of fat in the visceral fat and the ventral muscle were about 70 %.

Table VI. Mean concentrations and standard deviation of individual PCB components in different tissues of rainbow trout (*Salmo gairdneri*) given as mg/kg wet weight.

Comp. No.	Trunk muscle	Ventral muscle	Visceral fat	Liver
52	0.002 ± 0.001	0.020 ± 0.005	0.028 ± 0.005	0.001 ±<0.001
49	0.001 ±<0.001	0.007 ± 0.001	0.007 ± 0.007	<0.001
44	0.003 ± 0.001	0.026 ± 0.008	0.043 ± 0.004	0.002 ± 0.001
101	0.003 ± 0.001	0.035 ± 0.010	0.036 ± 0.008	0.001 ±<0.001
118	0.003 ± 0.001	0.035 ± 0.011	0.032 ± 0.007	0.001 ±<0.001
138	0.003 ± 0.001	0.042 ± 0.011	0.042 ± 0.007	0.002 ± 0.001
187	0.001 ±<0.001	0.006 ± 0.001	0.006 ± 0.001	<0.001
183	0.001 ±<0.001	0.005 ± 0.001	0.005 ± 0.001	<0.001
128	0.001 ±<0.001	0.006 ± 0.002	0.006 ± 0.001	<0.001
180	0.002 ± 0.001	0.022 ± 0.004	0.024 ± 0.005	0.001 ±<0.001
170	0.001 ±<0.001	0.007 ± 0.002	0.007 ± 0.002	<0.001
194	<0.001	0.002 ± 0.001	0.002 ± 0.001	<0.001

DISCUSSION

The importance of the food chain in the bioaccumulation of PCB´s by fish has been stressed by several authors (Borgmann and Whittle; 1983; Hilton et al., 1983; Thomann and Connolly, 1984). Thomann and Connolly (1984) reported that 99% of the PCB´s in lake trout came from food. A correlation between PCB´s in food and the concentration of PCB´s in cultured eel has also been found (Crisetig, Cattani and Roboni, 1982). The PCB levels and composition of individual PCB´s in the fish diet will be a major factor in determining the PCB levels and patterns in cultured fish.

Oil and meal from herring and capelin are important ingredients in commercially produced fish diets. The bioaccumulation of PCB´s in herring is well documented (Jensen et al.; 1972; Linko et al.,1974; Perttilä, Tervo and Parmanne, 1982; Musial and Uthe, 1983). Herring

from the Barent Sea showed a concentration of 20 ng/g wet weight muscle, whereas the concentration in the muscle of capelin ranged from 50 to 100 ng/g wet weight. The concentrations were based on the sum of components 52, 44, 95, 101, 110, 118, 153, 138, 128, 180, 170, and 194 (Palmork pers. com.). The components are listed in the order of elution. Values reported from commercial dry pellets ranged from not detectable to 300 ng/g on a dry weight basis (Mac, Nicholson and McCauley, 1979). Levels found in food given to cultured eel ranged from 30 to 135 ng/g wet weight (Crisetig et al., 1982).

A number of PCB components have so far been identified in fish (Zell et al., 1978; Ballschmiter et al., 1981; Tausch, Stehlik and Wihlidal, 1981; Duinker and Hillebrand, 1983; Tuinstra et al., 1983) and marine invertebrates (Duinker et al., 1983; Boon et al., 1985). Most of the quantified components found in highest concentrations in this study have also been reported as dominant components in the publications referred to. An exception was component 44. Component 44 has been found in fish in lower concentrations (Zell et al., 1978; Duinker and Hillebrand, 1983).. On other component found in lower concentrations, 128, was not reported found in fish samples in any of the referred publications. However, component 128 has been identified in marine invertebrates (Duinker et al., 1983; Boon et al.,1985).

PCB components are highly lipophilic (Bruggeman, Steen and Hutzinger, 1982), and the concentrations of PCB components in fish have been found to depend mainly on the lipid content (Lieb et al., 1974; Sugiura et al., 1979; Boon, 1985; Vuorinen et al., 1985). In this study no statistical differences were found between the concentration in the trunk muscle, ventral muscle and visceral fat when the concentrations were based on the lipid content. On a wet weight basis there were no statistical differences between the concentrations in the ventral muscle and the visceral fat, but the lipid content in this case was the same. Lower values were found in trunk muscle, which also had a much lower lipid content.

The sum PCB concentration and the concentration of the individual components were found to be lower in the liver compared to the other tissues. The same results were found by Lieb et al., (1974) and Guiney et al., (1977) in laboratory experiments, where rainbow trout were exposed to PCB´s. Liver samples from wild populations of salmon and

trout and one population of cultured rainbow trout were also found to have lower levels of PCB´s than muscle samples.

A number of different analytical methods and quantification methods have been used in the determination of PCB levels (Duinker et al., 1980). Use of capillary column gas chromatography, where individual components are identified, gives the most reliable estimates of the PCB concentrations (Anon, 1985). To achieve comparable data the methods or techniques of sampling, storage, extraction, concentration, isolation, identification and quantification of the investigated components should be similar (Palmork, 1986). In our study the congeners given by Duinker et al. (1987) were quantified and used to express the quantity of the PCB´s in the samples.

The concentration measured in the rainbow trout in this study appears to be much lower than the limits of 2 mg/kg in the edible portion of the fish given by the U.S. Food and Drug Administration.

We hope that for the future an International agreement will arise were results of analyses will be given as quantities of individual congeners or components only. It is important that the documentation of PCB analyses is given for the different congeners identified and quantified and that the "total" PCB concentration is given as the sum of the congeners agreed upon. As multidimensional gas chromatography will become more commenly used, the number of resolved congeners will increase and accordingly the number of reported congeners will also increase. If the recommendation that the concentration of the individual congeners should be reported is followed , then the given concentrations from different studies can be compared and on a time scale the concentrations of the individual congeners can be compared even if the number of identified congeners should increase.

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