Chlorinated Hydrocarbon Residues in Fish from Nové Mlýny Reservoirs Along Dyje River, Czech Republic

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Abstract

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The aim of this work was to monitor levels of chlorinated hydrocarbons in muscle of fish from Nové Mlýny reservoirs. Fish muscle was investigated for the content of DDT and its metabolites and polychlorinated biphenyls (Delor 106 and 7 indicator congeners of PCBs) in common carp (*Cyprinus carpio*, L.), bream (*Abramis brama,* L.) and pike perch (*Stizostedion lucioperca*, L.). Samples were collected and analysed by gas chromatography annually during 1995-2002. Residues of DDT and PCBs were found in all samples. The sum of DDT and its metabolites in fish muscle was determined in range of 0.001 to 0.672 $mg \cdot kg^{-1}$, Delor 106 in range of 0.047 to 0.476 $mg \cdot kg^{-1}$ and the sum of 7 indicator congeners of PCB in range of 0.001 to 0.133 $mg \cdot kg^{-1}$. DDT and PCB values did not exceed valid statutory limits of the Czech Ministry of Health except for a single case. The time course of DDT values was assessed. DDT values showed a significant decrease during the monitoring period. Decrease of DDT values was 91% for pike perch and 75% for bream. Significant correlations ($p < 0.05$) were found between concentrations of DDT and PCB and the content of fat in fish muscle. Our results confirm predominance of metabolite DDE and higher chlorinated congeners of PCB. Results are comparable with those of other authors.

DDT metabolites, PCB, fish muscle, common carp, bream, pike perch

Organochlorine contaminants (OCs) such as dichlorodiphenyl-trichloroethane (DDT) and polychlorinated biphenyls (PCBs) still belong among significant pollutants due to their low biodegradability and ability to accumulate in lipids. Fish is an important object for research of water ecosystem contamination by these substances due to its high position in food chain.

For decades, DDT was the most important insecticide worldwide. The main metabolites are DDD and DDE, of which the latter does not have a pesticide effect. The problems, such as resistance of many insect species, persistence and ability to accumulate in the lipid component of biota began to appear as soon as at the end of 1940´s. High acute toxicity of DDT-base insecticides for fish was documented (Mertlík and Svobodová 1973; Johnson and Finley 1980; Lakota et al. 1983).

PCBs like DDT belong among persistent contaminants. Due to their thermal stability, resistance to chemicals, high permittivity, flame-retardant properties, etc., they were widely used as cooling fluids, dielectrics in transformers, plasticizers in paints and varnishes, hydraulic fluids and heat transfer agents.

PCBs and DDT cause a variety of endocrine disrupting effects including estrogenic, anti-estrogenic and anti-androgenic effects (Kavlock et al. 1996; Keith 1997; You 2000). Due to this fact, monitoring of contamination level of PCBs and DDT is still relevant.

Systematic contamination monitoring of persistent organic pollutants was started in the Czech Republic at the end of 1970s. Contamination level was monitored in selected ponds, running waters, reservoirs and selected rainbow trout breeding ponds (Kredl et al. 1989; Svobodová et al. 1995, 2003, 2004; Hajšlová et al. 1997; Randák et al. 2001a, 2001b; Žlábek et al. 2002). The presented studies aimed attention to monitoring of DDT and its

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metabolites, HCH and HCB isomers, PCB both as sum of technical mixtures (Delor 103 and 106) and as sum of 7 indicator congeners of PCB. The results indicated higher contamination level in fish from running waters, fish with high content of fat and predatory fish species. Predominance of DDE metabolite and highly chlorinated congeners of PCB was indicated.

The aims of this work were:

1. to assess contamination level of DDT and PCBs in fish from Nové Mlýny reservoirs;
2. to compare contamination level among the individual reservoirs;
3. to compare contamination level among body sizes of fish;
4. to assess the course of DDT contamination level;
5. to compare the results with valid statutory limits of the Czech Ministry of Health (465/2002 Coll. and 53/2002 Coll.).

Materials and Methods

Location

Nové Mlýny reservoirs known also as Mušov Lakes (Fig. 1) have been situated in South Moravia (south-east part of the Czech Republic). There are three consecutively situated reservoirs (upper, middle and lower filled up in 1978, 1980 and 1989), the axis of which is represented by Dyje River. Dyje River is also the main inflow to the reservoirs. The other important sources are Svratka and Jihlava rivers, which flow into the middle reservoir. The basins of mentioned three rivers occupy rather large area. All three rivers run through important agriculture areas and important industry centres of South Moravia. Due to those facts, we can presuppose that contamination level of the reservoirs reflects contamination level of the basins.

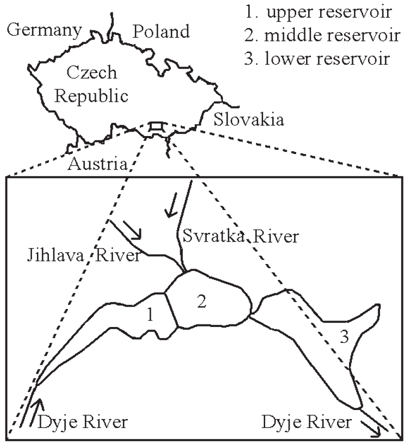

Fig. 1. Map of reservoirs under study position in the Czech Republic

Fish samples

Samples of common carp (*Cyprinus carpio,* L.), bream (*Abramis brama,* L.) and pike perch (*Stizostedion luciopera,* L.) were collected from Nové Mlýny reservoirs. One fish of each species was collected from each reservoir in 1995. In 1996-2002, samples of fish were divided into three length categories: 40–50 cm, 50–60 cm and above 60 cm for common carp and pike pearch and up to 25 cm, 25–35 cm and above 35 cm for bream. Four fishes from each length category were pooled to provide composite sample. Samples were collected annually from the middle reservoir during autumn period in 1995-2002 and from the lower and upper reservoirs in 1995–1999. Fish muscle was used for analyses. Samples were analyzed for the content of *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE; and expressed as a ΣDDT ($mg \cdot kg^{-1}$ of fish muscle), respectively ΣDDT ($mg \cdot kg^{-1}$ of fat), for the content of Delor 106 (in 1995–1998) and for the content of 7 indicator congeners (PCBs 28, 52, 101, 118, 138, 153, 180) expressed as a ΣPCB ($mg \cdot kg^{-1}$ of fish muscle or of fat).

Chemicals

Composite standard of PCB (PCB mix 3), composite standard of organochlorine pesticides (Pesticide mix 5) and matrix reference material R900100 (PCBs and organochlorine pesticides in pork fat) were used. The standards and reference material were obtained from Dr. Ehrenstorfer (Germany). Working solutions of analytes were prepared in n-hexane. All solvents used were of gas chromatography quality (Merck, CZ).

Analytical procedures

Gas chromatographic method was used for analysis of fish muscle samples. Analysis had the following steps: Representative part of fish muscle (50 g) was homogenized and extracted into diethylether (100 ml). Extract was dried by anhydrous sodium sulfate and evaporated in a rotary vacuum evaporator. An aliquot part of acquired fat portion (0.2 $\pm$ 0.05 g) was dissolved in n-hexane (2 × 4 ml) and cleaned up on Florisil packed column. Cleaned solution was concentrated by rotary vacuum evaporator into 2 ml and used for analysis. Gas chromatograph Hewlet Packard 5890 series II with electron capture detector (ECD) and MDN-5S (60 m) column was used for determination of analytes. HP 3365 ChemStation series II software was used for evaluation of chromatograms. Determinableness limit of DDT, its metabolites and individual indicator congeners of PCB is 0.0001 $mg \cdot kg^{-1}$ of fish muscle for each analyte. Expanded uncertainty is 8% on condition that coefficient of expansion is k = 2.

Evaluation of the results

DDT and PCB dates were evaluated statistically using linear regression and log-linear regression (MS Excel 97). The analysis of variance (ANOVA) was used for testing species, reservoirs and fish length differences (Statgraphics soft.). Decrees of the Czech Ministry of Health No. 465/2002 Coll. and 53/2002 Coll. and the Acceptable Daily Intake (ADI) value assigned by WHO were used in order to interpret of results.

Results and Discussion

DDT and its metabolites

Residues of ΣDDT and its metabolites were detected in all analysed samples (Table 1). Values of ΣDDT did not exceed valid statutory limit (0.5 $mg \cdot kg^{-1}$ of fish muscle) of the Czech Ministry of Health (465/2002 Coll.) except for one case (composite sample of bream, length category above 35 cm, year 1997, lower reservoir).

No significant differences in ΣDDT values were observed among the individual reservoirs ($p < 0.01$). Presumption of higher values from the middle and lower reservoir because of inflowing Jihlava and Svratka rivers into the middle reservoir was not confirmed. Due to this fact, monitoring has been reduced to the middle reservoir since 2000.

ΣDDT values expressed in $mg \cdot kg^{-1}$ of fish muscle were significantly higher in bream ($p < 0.01$) in comparison with pike perch. The other differences among species were not statistically significant. ΣDDT values expressed in $mg \cdot kg^{-1}$ of fat were significantly higher in pike perch ($p < 0.01$) in comparison with common carp. There were not significant differences in ΣDDT values expressed in $mg \cdot kg^{-1}$ of fat between bream and common carp.

No significant differences ($p < 0.01$) were found in ΣDDT values among length categories in $mg \cdot kg^{-1}$ of fish muscle and $mg \cdot kg^{-1}$ of fat as well. This fact was in contradiction to findings of other authors (Harding et al. 1997; Olsson et al. 2000; Ueno et al. 2002).

Linear regression was used for testing of relationship between ΣDDT values in $mg \cdot kg^{-1}$ of fish muscle and the content of fat acquired during 1996–1999. Results of all length categories and all three reservoirs were used for testing. Results were tested separately for each year and species. Significant correlation ($p < 0.05$, n = 9) was found for all three species, except for a single case (carp, 1999). Correlation coefficients (1996-1999) were for bream r = 0.873, 0.963, 0.895, 0.946, for pike perch r = 0.963, 0.991, 0.851, 0.952 and for carp r = 0.699, 0.965, 0.858, 0.582. This fact ascribes the reason why significant differences among length categories were not found. Contamination level depended primarily on content of fat but not on the length of fish due to variations in fatness. These results are in agreement with results of other authors (Niimi and Oliver 1989; Hajšlová et al. 1997; Svobodová et al. 2003, 2004).

The proportion of DDT and its metabolites in the fish collected during 1999–2002 show predominance of *p,p'*-DDE (75.9%) followed by *p,p'*-DDD (15.6%), *p,p'*-DDT (5.4%) and *o,p'*-DDT (3.1%). Predominance of *p,p'*-DDE metabolite confirmed fact that present contamination level is of previous origin due to slow biotransformation of DDT.

Values of DDT and its metabolites in the analysed samples of fish fluctuated among the monitoring period by reason of difference of fish fatness among the years. It is probably caused by various food supplies in the individual years.

Table 1. Contents of DDT and its metabolites, Delor 106 (1995-1998) and 7 indicator congeners of PCB (1999-2002) in pike perch, bream and common carp. Values of organochlorine contaminants residues are expressed in mg·kg^{-1} of fish muscle

ΣDDT [mg·kg^{-1} of fish muscle]									
year	common carp 40 –50 cm	common carp 50 - 60 cm	common carp above 60 cm	pike perch 40 – 50 cm	pike perch 50 – 60 cm	pike perch above 60cm	bream up to 25 cm	bream 25 - 35 cm	bream above 35 cm
upper reservoir									
1995		0.088			0.085			0.305	
1996	0.088	0.061	0.053	0.023	0.028	0.028	0.048	0.090	0.247
1997	0.037	0.062	0.066	0.013	0.029	0.018	0.083	0.095	0.243
1998	0.210	0.170	0.060	0.110	0.030	0.090	0.120	0.060	0.150
1999	0.011	0.012	0.008	0.013	0.021	0.014	0.024	0.012	0.014
middle reservoir									
1995		0.261			0.118			0.268	
1996	0.025	0.063	0.033	0.132	0.125	0.167	0.083	0.211	0.051
1997	0.027	0.048	0.110	0.024	0.027	0.025	0.105	0.145	0.243
1998	0.180	0.110	0.360	0.040	0.080	0.020	0.050	0.180	0.040
1999	0.006	0.028	0.008	0.008	0.001	0.005	0.014	0.019	0.064
2000	0.109	0.136	0.093	0.035	0.030	0.062	0.116	0.051	0.054
2001	0.007	0.005	0.107	0.007	0.033	0.014	0.072	0.011	0.065
2002	0.023	0.032	0.379	0.007	0.010	0.012	0.087	0.027	0.107
lower reservoir									
1995		0.272			0.115			0.226	
1996	0.033	0.008	0.117	0.057	0.025	0.032	0.052	0.230	0.124
1997	0.037	0.367	0.326	0.203	0.084	0.052	0.092	0.495	0.672
1998	0.060	0.020	0.050	0.100	0.070	0.040	0.080	0.090	0.070
1999	0.008	0.010	0.029	0.004	0.008	0.009	0.019	0.015	0.026
Delor 106 (1995 – 1998) and ΣPCB (1999 – 2002) [mg·kg^{-1} of fish muscle]									
upper reservoir									
1995		0.135			0.067			0.473	
1996	0.274	0.307	0.301	0.064	0.087	0.126	0.379	0.257	0.432
1997	0.144	0.342	0.250	0.066	0.136	0.097	0.293	0.258	0.323
1998	0.090	0.320	0.110	0.420	0.160	0.300	0.230	0.080	0.240
1999	0.004	0.004	0.002	0.006	0.007	0.004	0.011	0.004	0.004
middle reservoir									
1995		0.216			0.155			0.152	
1996	0.089	0.179	0.129	0.289	0.440	0.405	0.245	0.470	0.164
1997	0.125	0.173	0.273	0.047	0.116	0.099	0.298	0.168	0.196
1998	0.070	0.350	0.220	0.120	0.350	0.440	0.180	0.380	0.210
1999	0.027	0.012	0.006	0.003	0.001	0.002	0.005	0.007	0.019
2000	0.060	0.052	0.044	0.017	0.014	0.027	0.032	0.023	0.025
2001	0.001	0.001	0.032	0.002	0.002	0.006	0.033	0.003	0.002
2002	0.007	0.010	0.133	0.002	0.003	0.004	0.016	0.008	0.028
lower reservoir									
1995		0.072			0.161			0.159	
1996	0.204	0.074	0.243	0.050	0.068	0.072	0.187	0.397	0.349
1997	0.142	0.476	0.393	0.410	0.348	0.160	0.206	0.389	0.468
1998	0.180	0.060	0.210	0.340	0.120	0.190	0.090	0.290	0.170
1999	0.002	0.003	0.008	0.003	0.002	0.002	0.009	0.004	0.007

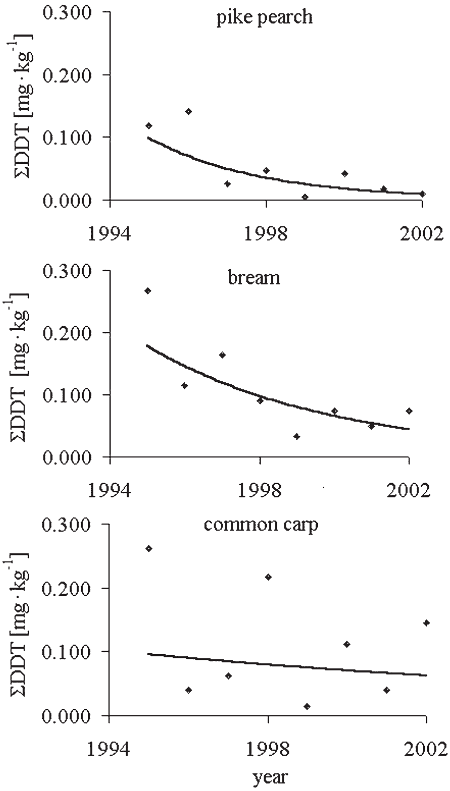

Fig. 2. Time course of DDT contamination level expressed in mg/kg of fish muscle

The time course of DDT contamination level expressed in mg·kg^{-1} of fish muscle for the individual species is shown in Fig. 2. Time course was assessed only for the middle reservoir, the only one for which we had results for sufficiently long period of time. Results of three length categories were used for assessment of time course for the individual species. Results from 1995 were included into assessment of time course for extended period of time acquisition. Simple log-linear regression analysis was carried out in order to detect time course. Decrease of contamination level in 1995–2002 was calculated from regression equations and expressed in percentages. The greatest decrease was found for pike perch (91%) followed by bream (75%) and common carp (35%). Great annually fluctuation of the results can be seen for common carp. Stocking of common carp into reservoirs at the age of 2–3 years probably causes this, therefore common carp cannot be taken as an indicator fish reflecting contamination level of Nové Mlýny reservoirs. Results of common carp also showed relatively the worst correlation between ΣDDT values in mg·kg^{-1} of fish muscle and content of fat.

The authors realize that given monitoring time and number of dates do not enable to determine time course of contamination level exactly. Nevertheless we could say contamination level significantly decreased over monitoring time for bream and pike perch. Predominance of *p,p'*-DDE metabolite also confirmed degradation of DDT in water ecosystems of Nové Mlýny reservoirs. Hajšlová et al. (1997) studied contamination level of DDT in Nové Mlýny reservoirs too. They presented mean values of DDT and its metabolites in common carp 0.083 mg·kg^{-1} of fillets, in bream 0.167 and in pike perch 0.027 and indicated predominance of *p,p'*-DDE metabolite. It corresponds to our findings.

There are not literature data enough to compare time courses of DDT contamination levels in the Czech Republic. Only Randák et al. (2001ab) and Žlábek et al. (2002) presented a comparison of results from three ponds in South Bohemia among years 1991, 1992 and 1999. Their results showed low increase of contamination level.

The ADI value as assigned by WHO for DDT and its metabolites is 0.020 mg·kg^{-1}of body weight·day^{-1}. The mean of values obtained in 1999–2002 from the middle reservoir of all three species was used for evaluation of the hygienic risk. A 70 kg-man should have to consume 27.5 kg of fish meat per day to achieve the ADI for DDT and its metabolites. The mean yearly freshwater fish consumption in the Czech Republic amounts to 1 kg per capita (Berka 1998), which represents 2.74 g·day^{-1}. It amounts to 0.01% of ADI when assuming consumption of the tested fish species from the middle reservoir.

PCB

Residues of PCBs were detected in the all analysed samples (Table 1). Values of 7 indicator congeners of PCB did not exceed valid statutory limit (2 mg·kg^{-1} of fish muscle) of the Czech Ministry of Health (53/2002 Coll.) and were for 2 or 3 numerical orders of magnitude under limit. Delor 106 values cannot be compared with valid statutory limit because it was determined as sum of technical mixtures Delor 103 and 106 and was 0.5 mg·kg^{-1} of fish muscle (Directive No. 50/1978 "Contaminants in food"). Nevertheless, Delor 106 values did not exceed this limit.

No significant differences in Delor 106 and ΣPCB values were observed among the individual reservoirs ($p < 0.01$) and presumption of higher values from the middle and lower reservoirs were not confirmed. Monitoring has been also reduced to the middle reservoir since 2000.

No significant differences ($p < 0.01$) were found in Delor 106 and ΣPCB values among the length categories in mg·kg^{-1} of fish muscle and mg·kg^{-1} of fat as well and among the species in mg·kg^{-1} of fish muscle.

Delor 106 and ΣPCB values expressed in mg·kg^{-1} of fat were significantly higher ($p < 0.01$) in pike perch in comparison with common carp and bream. There were no significant differences ($p < 0.01$) in Delor 106 and ΣPCB values expressed in mg·kg^{-1} of fat between bream and common carp.

Significant correlation ($p < 0.05$, n = 9) between Delor 106 (1996-1998) and ΣPCB (1999) contents and the content of fat was found for all three species except results obtained in 1998. Correlation coefficients (1996-1999) were for bream r = 0.718, 0.675, 0.650, 0.865, for pike perch r = 0.952, 0.916, 0.362, 0.889 and for carp r = 0.930, 0.926, 0.032, 0.687.

Following proportions of 7 indicator congeners of PCB in fish collected during 1999–2002 from the middle reservoir could be presented: PCB153 = 29.8%, PCB138 = 21.6%, PCB180 = 18.8%, PCB101 = 13.2%, PCB28 = 10.0%, PCB52 = 4.3% and PCB118 = 2.4%. Results show predominance of highly chlorinated congeners. We cannot indicate time course of PCB contamination level because we do not have data for sufficiently long period of time.

Hajšlová et al. (1997) also studied PCB contamination level in Mušov Lakes. They presented mean values of 7 indicator congeners of PCB in common carp 0.0345 mg·kg^{-1} of fillets, in bream 0.0749 and in pike perch 0.0095 and indicated predominance of high chlorine congeners. It corresponded to our findings.

The ADI value as assigned by WHO for sum of 7 indicator congeners of PCB is 0.005 mg·kg^{-1}of body weight per day. The mean of values obtained in 1999–2002 from the middle reservoir of all three species was used for evaluation of the hygienic risk. A 70 kg-man should have to consume 18.8 kg of fish meat per day to achieve the ADI for 7 indicator congeners of PCB. The mean yearly freshwater fish consumption in the Czech Republic amounts to 0.015% of ADI when assuming consumption of the tested fish species from the middle reservoir.

Rezidua chlorovaných uhlovodíků ve svalovině ryb z nádrží Nové Mlýny na řece Dyji

Cílem této práce bylo monitorovat stav zatížení ryb organochlorovými sloučeninami v nádržích Nové Mlýny na řece Dyji. Množství pesticidu DDT a jeho metabolitů a polychlorovaných bifenylů bylo sledováno ve svalovině kapra obecného (*Cyprinus carpio,* L.), cejna velkého (*Abramis brama,* L.) a candáta obecného (*Stizostedion luciopera,* L.). Vzorky byly odebírány každoročně v rozmezí let 1995-2002. K analýzám byla použita metoda plynové chromatografie. Rezidua DDT a PCB byla nalezena ve všech vyšetřených vzorcích. Obsah DDT a jeho metabolitů se ve svalovině ryb pohyboval v rozmezí od 0,001 do 0,672 mg·kg^{-1}, Delor 106 v rozmezí od 0,047 do 0,476 mg·kg^{-1} a suma 7 indikátorových kongenerů PCB v rozmezí od 0,001 do 0,133 mg·kg^{-1}. Hodnoty ΣDDT

a ΣPCB až na jeden případ nepřekročily hygienický limit stanovený vyhláškami Ministerstva zdravotnictví č. 465/2002 Sb a 53/2002 Sb. U hodnot DDT byl hodnocen trend zatížení. Byl zaznamenán významný pokles kontaminace DDT, a to u candáta obecného o 91% a u cejna velkého o 75 %. Byla zjištěna významná korelace ($p < 0.05$) mezi hodnotami DDT, PCB a obsahu tuku. Výsledky potvrdily převahu metabolitu *p,p*'-DDE a vícechlorovaných kongenerů PCB. Zjištěná úroveň kontaminace je srovnatelná s výsledky jiných autorů z lokalit České republiky.

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