Water Air Soil Pollut (2009) 197:193–209
DOI 10.1007/s11270-008-9803-z

Age-Dependent Bioaccumulation of Organochlorine Compounds in Fish and their Selective Biotransformation in Top Predators from Lake Maggiore (Italy)

Pietro Volta · Paolo Tremolada · Maria Chiara Neri · Gianluigi Giussani · Silvana Galassi

Received: 29 December 2007 / Accepted: 15 July 2008 / Published online: 24 August 2008

Abstract Fish and piscivorous bird eggs collected in 2003 from Lake Maggiore (Italy), were analysed for PCB and DDT contamination. Lake Maggiore has been severely polluted by DDT through production of the pesticide within its catchment. Although agricultural application of DDT was banned in Italy in the 1978, industrial production continued until 1996, with enough contamination of water and soil for serious bioaccumulation in the lake biota. PCB and DDT concentrations in a whitefish (*Coregonus macrophthalmus* Nusslin 1882) were seen to be dependent on season and fish age, but not on sex. The average increase of the lipid-normalised concentration of DDTs and PCBs was two-fold across season and also across age, resulting in an overall increase of four fold. The seasonal variation was related to the eco-physiological cycle of the fish and to the contamination dynamic of the lake, while the effect of the fish age was explained on the base of biomagnification-related mechanisms. A fugacity model was applied to predict the age-dependent bioaccumulation potential of PCBs, whose concentrations were rather stable in recent years in the lake. Predicted values for compounds with negligible biotransformation were in good agreement with experimental data (calculated *vs*. experimental mean difference of 14%), and a relationship between the increase of experimental age-dependent concentration and K_{ow} was observed. The good correspondence between the predicted and the measured values for most PCB congeners confirmed the general inability of fishes to biotransform these compounds. On the contrary, the importance of biotransformation processes was recognised in birds; eggs of a fish eating bird (*Podiceps cristatus*) from the same area selectively bioaccumulated *p*,*p*′-DDE. For PCBs, congener 149 appears to be completely metabolized by the bird species, and congeners 95, 101, 132, 151 and 174 were reduced as well. The role of the *meta–para* free position on at least one phenyl ring of PCB congeners in biotransformation processes was confirmed.

Keywords Food chain · Biotransformation · Fish lipids · Distribution model · Whitefish · *Coregonus* sp.

P. Volta (✉) · G. Giussani
Institute of Ecosystem Study, National Research Council,
Largo Tonolli, 50,
Verbania–Pallanza, VB 28922, Italy
e-mail: p.volta@ise.cnr.it

P. Tremolada · M. C. Neri · S. Galassi
Department of Biology, University of Milan,
Via Celoria 26,
Milan 20133, Italy

1 Introduction

The extent to which organochlorine compounds (OCs) impact top predators, man included, is not completely known (Van den Berg et al. 1998; Rogan

and Chen 2005). OC pattern and the levels of individual pollutants may greatly change as functions of the amount introduced into the environment, climate and aquatic community species composition. Moreover, the extent of accumulation differs from species to species due to differences in diet and metabolic pathways (Drouillard et al. 2001). Additionally within a species, there may be variability in accumulation rates related to age, season, sex and the physiological condition of the animal (Nakata et al. 1995).

The aim of this paper is to analyse this additional variability within a pelagic fish, the whitefish (*Coregonus macrophthalmus* Nusslin 1882) and the possible biotransformation processes occurring in the pelagic food web. The extent of these phenomena greatly affects bioaccumulation according to the concept of 'selective bioaccumulation' (Guruge and Tanabe 1997; Henriksen et al. 2000). Although the mechanisms and the extent of bioaccumulation are mainly known (Gobas et al. 1988, Clark et al. 1990; Gobas et al. 1993) and have been kinetically measured (Drouillard et al. 2001; Paterson et al. 2007), the role of biotransformation is more complex and incompletely understood even for such well-known compounds as DDTs and PCBs (UNEP 2001).

The environmental transformation of DDT, whose commercial product contains up to 85% of *p*,*p*′-DDT and up to 20% of *o*,*p*′-DDT, with minor proportions of DDD (both *p*,*p*′- than *o*,*p*′-) and other constituents (ATDSR 2000), is well known. The metabolites/parent compound ratio is considered a measure of the 'age' of the contamination (Calamari et al. 1994), and among the metabolites, *p*,*p*′-DDD is known to be preferentially formed in the anaerobic condition of sediments while *p*,*p*′-DDE is formed through aerobic metabolism. Since the late 1970, when the agricultural use of DDT was banned in most industrialized countries, temporal changes in the metabolite/parent compound ratio have been observed in many lentic environments, such as the Great Lakes (de Vault et al. 1996; Renaud et al. 1999; Marvin et al. 2003). In Lake Maggiore, which received the wastewaters of a DDT plant up until 1996, the DDT relative composition in whitefish showed a 50% reduction in the proportion of the parent compound from 1998 to 2003, the transformation being mostly to *p*,*p*′-DDD metabolite (CIPAIS 2005). In 2003, the metabolite/parent compound ratio in surface sediment was around 20 (CIPAIS 2005).

PCBs are a mixture of homologues and isomers produced with different degrees of chlorination in many countries; in Italy mainly Fenclor 54 and 60 mixtures (corresponding to Aroclor 1254 and 1260) were produced and used (Breivik et al. 2002). In animals several transformation processes have been described. Generally much lower biotransformation rates were observed in fish than in birds and mammals (Boon et al. 1989; Buckman et al. 2004). On the basis of the substitution pattern, three groups of congeners were proposed by Boon et al. (1989): those without a free chlorine *meta–para* position and more than 1 *ortho*-Cl (persistent congeners); those with a free chlorine *ortho–meta* position on at least one of phenyl rings, and no more than 1 *ortho*-Cl (potentially biotransformed); and those with a free chlorine *meta–para* position on at least one of phenyl rings, and more than 1 *ortho*-Cl (readily biotransformed). Studies in birds showed that PCB congeners containing a vicinal *meta–para* hydrogen substituent on at least one phenyl ring can be classified as 'readily cleared' while 'slowly cleared' are those congeners hindered at these positions (Bush et al. 1974; Walker 1990; Guruge and Tanabe 1997; Drouillard et al. 2001). Tanabe et al. (1988) introduced a metabolic index (MI) in order to assess metabolic susceptibility for each PCB compound relative to congener 180 based on the concentration ratio in the diet to that in the organism. Several PCB congeners, such as PCB 153, are highly recalcitrant to metabolic attack; for them the major mechanism of whole body elimination in rats has been demonstrated to be fecal egestion of the parent compounds (diffusive losses) (Wyss et al. 1982; Matthews and Andersen 1975).

In this study, an Italian lake (Lake Maggiore) recently polluted by DDT of industrial origin (Solcà et al 2006), was chosen for as case study to understand the influence of season, age and sex on the PCB and DDT bioaccumulation phenomena in whitefish. Concentrations were determined in fish tissues and gonads. The OC profiles measured in fish were then compared with those determined in the eggs of a piscivorous bird, the great crested grebe (*Podiceps cristatus*), living in the same area. OC data in bird eggs were taken from CIPAIS (2005). We believe that changes observed in predator–prey relationships, as well as among age classes could provide very useful indications of the metabolic transformations that are

related to detoxification processes and/or production of possible toxic metabolic intermediates.

2 Materials and Methods

Sampling Fish sampling was carried out in April, July and November 2003, in a central area of Lake Maggiore (Northern Italy). Benthic and pelagic gill nets (mesh size between 32 and 55 mm knot to knot) were set from late afternoon (5 P.M.) to the following morning (8 A.M.). Fish were measured (total length and total weight) and sexed by scale reading. Six fish for each age class (from 1 to 6 years) and for each sex were selected. Furthermore, mature gonads were taken from ten males and ten females captured in November. Samples were stored at −20°C until the preparation step was performed. Sample characteristics are reported in Table 1.

Sample Preparation Fish tissue was taken from the dorsal portion of the fish, above the lateral line between the head and the dorsal fin. The tissues of six fish of the same age and sex were pooled together and homogenized by a steel mixer in order to obtain a single pooled sample. Similarly, a pool of ten fish gonads for each sex was homogenized. All pooled samples (tissues and gonads) were kept at −20°C until they were lyophilized in an Edwards Minifast Mod. 01 freeze dryer.

Chemical Analysis Lipid content was determined gravimetrically after Soxtec extraction (PBI International) with petroleum ether in triplicate. A subsample of about 2 g was extracted in Soxhlet with *n*-hexane-acetone 1:1 (Carlo Erba, Pesticide grade) for OC determination. After the extraction, the samples were concentrated by rotary evaporation to dryness and then re-suspended in 5 ml of *n*-hexane. The same volume of concentrated sulphuric acid (95–97%, J.T. Backer, Deventer, The Netherlands) was added to the extract and left to react for 24 h. The hexane layer was cleaned on a Florisil column (4×0.7 cm Florisil). The sample was eluted with 25 mL of *n*-hexane:

Table 1 Sex, age, season, dry weight (dw) and lipid content on the wet weight basis (% wet weight) of the analysed *C. macrophthalmus* samples

Sample	Type	Sex	Age	Season	dw (%)	Lipid (% ww)
M3-Apr	Fish tissues	M	3	Apr	28.1±1.0	8.0±0.3
M4-Apr	Fish tissues	M	4	Apr	37.1±0.9	9.5±0.3
F3-Apr	Fish tissues	F	3	Apr	31.4±2.0	9.2±0.6
F4-Apr	Fish tissues	F	4	Apr	32.5±2.7	9.3±0.8
M1-Jul	Fish tissues	M	1	Jul	35.8±3.0	12±1.0
M2-Jul	Fish tissues	M	2	Jul	37.8±5.5	12±1.8
M3-Jul	Fish tissues	M	3	Jul	37.9±3.5	12±1.1
F2-Jul	Fish tissues	F	2	Jul	28.1±6.7	8.9±2.1
F3-Jul	Fish tissues	F	3	Jul	33.4±11	11±3.6
F4-Jul	Fish tissues	F	4	Jul	35.1±1.5	11±0.6
M1-Nov	Fish tissues	M	1	Nov	26.3±1.5	7.8±0.5
M2-Nov	Fish tissues	M	2	Nov	33.6±5.9	10±1.8
M3-Nov	Fish tissues	M	3	Nov	21.2±1.8	10±0.4
M4-Nov	Fish tissues	M	4	Nov	31.7±2.0	10±0.6
F1-Nov	Fish tissues	F	1	Nov	24.7±0.8	7.2±0.2
F2-Nov	Fish tissues	F	2	Nov	30.1±3.0	9.2±0.9
F3-Nov	Fish tissues	F	3	Nov	40.8±6.2	13±2.0
F4-Nov	Fish tissues	F	4	Nov	38.0±3.7	13±1.3
F5-Nov	Fish tissues	F	5	Nov	33.2±1.1	9.9±0.5
F6-Nov	Fish tissues	F	6	Nov	39.7±5.7	12±1.8
Sperm	Sperm			Nov	7.5±0.8	2.9±0.3
Eggs	Eggs			Nov	14.6±0.9	4.4±0.2

Dry weight and lipid content values are followed by ±standard deviation values.

dichloromethane 85:15 *v*/*v* and adjusted to a volume suitable for analysis. Cleaned extract (1 μl) was injected into a Fison Top 8000 gas-chromatograph equipped with a ^{63}Ni Electron Capture Detection (ECD), and with an on-column injector. A capillary column CP-Sil-8 CB, 50 m×0.25 mm I.D., film thickness 1.2–2 μm was used with a temperature program from 60°C to 180°C at 20°C min^{-1}, followed by a run from 180°C to 250°C at 3°C min^{-1}, and a further run from 250°C to 270°C at 2°C min^{-1}. Helium was used as the carrier gas (at 0.7 mL min^{-1}), and nitrogen was used as the auxiliary gas for the detector at about 30 ml min^{-1}. The detector temperature was fixed at 330°C.

The following IUPAC congener numbers in elution order were screened: 95, 101, 151, 149, 118, 132, 153, 138, 187, 183, 174, 177, 170, 180, 195, 201, 203, 194 and 206. The following DDT compounds were analysed: *p*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD, *o*,*p*′-DDT, *o*,*p*′-DDE, *o*,*p*′-DDD. Quantitative determination was performed as described in Bettinetti et al. (2006). Good laboratory practices were tested on a reference material (lyophilized mussels) kindly provided by IAEA (Monaco). Mean recoveries were greater than 80% for PCBs and DDTs with a mean variability of around 10%. The detection limit was 0.2 and 0.5 ng g^{-1} dry weight for PCB congeners and DDT compounds, respectively.

Brief theory of the modelling approach adopted In order to model the bioaccumulation potential of lipophilic compounds in fish of different age, the fugacity approach of Campfens and Mackay (1997) was adopted. Overall contaminant uptake from water and food is described as

$$f_f = f_w \times D_w/(D_w + D_e + D_m + D_g) + f_a \times D_a/(D_w + D_e + D_m + D_g) \quad (1)$$

Where: f_f, f_w and f_a are the fugacity values in Pa for fish, water and food respectively; D_w and D_a are the *D*-values (flux parameters in moles per hour per Pascal) for exchange with water and intake from food respectively; D_e, D_m, and D_g are the *D*-values (flux parameters in moles per hour per Pascal) for the losses for egestion, metabolism and growth dilution respectively.

As *C. macrophthalmus* is a pelagic fish feeding on zooplankton, the assumption of an equal-fugacity between water and fish food was introduced ($f_a \approx f_w$) according to Campfens and Mackay (1997). If so, Eq. 1 can be simplified as:

$$\frac{f_f}{f_w} = D_w/(D_w + D_e + D_m + D_g) + D_a/(D_w + D_e + D_m + D_g) \quad (2)$$

If steady state conditions can be assumed, different lipid-normalised concentrations in fish of different age can be due to differences in their bioaccumulation capability. In these conditions, considering fishes of different age, the ratio of their lipid-normalized concentrations must be equal to the ratio of their fugacity and mathematically to the ratio between their fugacity and water

$$\frac{C_{fn}}{C_{f1}} = \frac{f_{fn}}{f_{f1}} = \frac{\frac{f_{fn}}{f_w}}{\frac{f_{f1}}{f_w}} \quad (3)$$

Where: C_{fn} and C_{f1} are the lipid-normalized concentrations of fish '*n*' year old and 1 year old; f_{fn} and f_{f1} are the fugacity of fish '*n*' year old and 1 year old; by Eqs. 2 and 3, the relative increase of the lipid-normalized concentrations in fish between the year '*n*' and the year 1 is equal to the ratio between their *D*-values describing their input from water and food, as follows

$$\frac{C_{fn}}{C_{f1}} = \frac{D_{wn}/(D_{wn} + D_{en} + D_{mn} + D_{gn}) + D_{an}/(D_{wn} + D_{en} + D_{mn} + D_{gn})}{D_{w1}/(D_{w1} + D_{e1} + D_{m1} + D_{g1}) + D_{a1}/(D_{w1} + D_{e1} + D_{m1} + D_{g1})} \quad (4)$$

The sub-script '*n*' and '1' in Eq. 4 indicate the age of the fish to which concentrations and flux parameters refer. For a detailed description and definition on what *D*-values are and how they were calculated, the authors refer back to the publication of Campfens and Mackay (1997). In the calculations of the *D*-values for fish of different age specific biological data of *C. macrophthalmus* were considered (Table 2).

Table 2 Age specific physiological parameters considered for the model application

Organism	Age class	Wet weight (g)	Lipid fraction	Growth rate (day^{-1})	Consumption rate ($g\ g^{-1}\ day^{-1}$)	Assimilation efficiency
Zooplankton		0.001	0.02	0.04	0.3	0.6
Whitefish	1	100	0.08	0.00274	0.03	0.6
	2	207	0.09	0.001416	0.03	0.6
	3	307	0.1	0.000893	0.03	0.6
	4	378	0.11	0.000515	0.03	0.6
	5	433	0.12	0.000348	0.03	0.6
	6	478	0.13	0.000258	0.03	0.6

Data for zooplankton and consumption rate and assimilation efficiency for whitefish are taken from Connolly and Pedersen (1988). Wet weight lipid fraction and growth rate for whitefish were derived from the experimental values of the fish analysed in this work and from others previously caught by the same authors.

As in the case of biomagnification (Campfens and Mackay 1997), age-dependent bioaccumulation occurs mainly when uptake from food dominates on the uptake from water ($D_a > D_w$). A second condition for having an increase of the concentrations with the fish age is that D_a and thus D_e (D_e is related to D_a by the equation: $D_e = D_a/Q$ where Q is the maximum or limiting biomagnification factor, typically about 3 as reported by Mackay 2001) will be more important than $D_m + D_g$, otherwise the major contaminant intake in fish of increasing size will be hidden by the higher metabolization capacity or dilution capacity of fish of increasing mass. The age-dependent bioaccumulation (increase of the lipid-normalized concentrations with the fish age) can be view as a combination of an increase of the food intake (increase of D_a) and a decrease of the growth dilution (decrease of D_g) for fishes of increasing age, and it can occur when D_a and thus D_e are more important than D_w, D_m and D_g. In other words, if losses by respiration, metabolism and growth dilution will become predominant on the food intake process, the age-dependent bioaccumulation do not occur. In particular an high biotransformation rate can reduce the contamination level in the animal as well as the age-dependent bioconcentration phenomenon. By this assumption the age-dependent bioconcentration can be view as an indication of a low biotransformation rate in the animal. Following the same approach of Arnot et al. (2008), biotransformation rate constants can be calculated as the difference between two quantities, a field-measured increases of the lipid-normalised concentrations of fish of different age and a model-derived age-dependent bioaccumulation factor estimated assuming no biotransformation. Because biotransformation reduces the age-dependent bioaccumulation factor, a very low biotransformation rate, e.g. a half-life of 5,000 days, or the absence of this output can be adopted to obtain for each chemical the maximum age-dependent bioaccumulation potential of a given compound (modelled-derived maximum age-dependent bioaccumulation factor). On the other hand, the field-measured ratio of the lipid-normalised concentrations of fish of different age will give an experimental bioaccumulation potential that includes biotransformation. In this way, the comparison between the maximum age-dependent bioaccumulation potential (calculated value without biotransformation) and the field-measured one (with biotransformation) can give a measure of the intensity of degradation processes under field conditions.

3 Results and Discussion

3.1 Lipid Trend

The lipid content is a trace variable for the ecophysiology of the organism and it is a basic variable for the comprehension of the distribution of the considered contaminants, which preferentially partition into fat tissues. The lipid content in whitefish samples (Table 1) was analysed and related to sex and season as factors and to age as a covariate. Fish collected during different periods of the year had statistically different lipid content ($P=0.023$). The same is true for fishes of different ages ($P=0.016$),

while sex (P=0.59) does not explain any variability. The mean percentages of lipids, adjusted for age as a covariate, were 8.7, 11.5 and 10.1 based on wet weight in April, July and November, respectively. A seasonal cycle of the lipid content is expected in *C. macrophthalmus* due to its eco-physiology (Berg & Grimaldi 1965; Giussani & de Bernardi 1977). Fat is stored roughly between April and September, then mobilized to basic metabolism and gonads development in October–March; gametes are spawned in January–February. The increase of lipids with fish age is a common behaviour in fishes and can be related to the greater feeding ability of older fishes and to the decreasing growth rate with the age (mean lipid fraction and growth rate in whitefish from Lake Maggiore are reported in Table 2). Between age class 1 and 6 the lipid content increases of nearly 50%, while the growth rate decreases of one order of magnitude.

Since lipid changes during the year and among the age classes are expected to cause differences in the contamination burden, OC concentrations in whitefish were normalized on the lipid base (Table 3).

3.2 Season, Sex and Age Effect

PCB and DDT concentrations, expressed on a lipid basis, were subjected to statistical analysis for sex and season as factors and for age as a covariate (Table 4). Significant differences (P<0.05) were found between samples collected at different times of the year (season effect) and between fish of different age (age effect). By contrast, sex had no impact (P>0.14).

The mean PCB and DDT concentrations, adjusted for age as a covariate, reveal a very similar seasonal trend (Fig. 1), characterized by a minimum in the summer and a slight increase in the autumn. The highest concentration was measured in April when lipid content was minimum. Paterson et al. (2007) showed that in the over-winter period lipid reserves in yellow perch (*Perca flavescens*) decreased consistently while no loss of PCBs occurred. PCBs body burden was redistributed during lipid mobilisation with a consequent increase of lipid-normalised concentrations. The depletion of lipid associated with pre-spawning migration of sockeye salmon (*Oncorhynchus nerka*) causes an increase of PCB, PCDD and PCDF concentrations (Debruyn et al. 2004). In whitefish the overall increase of DDT and PCB concentrations from July to April is around two-fold, while the lipid reduction is around 25%. Our observed lipid depletion cannot explain alone the concentration increase observed in early spring (April) in comparison to the levels reached in summer. Other elements must be taken into account. In April whitefish already started to feed on newly growing plankton that can be thought to be more contaminated than in summer because of the typical contamination cycle of the lake. In Lake Maggiore, a pollution peak was usually observed in winter in the epilimnion waters, at the end of the stratification period (Bettinetti et al. 2006) then, in summer, the contamination levels decreased for two reasons: higher volatilisation losses occurring in summer (Jeremiason et al. 1994) and efficient transport of contaminants from surfaces waters to benthic regions by settling particles (Baker et al. 1991; Axelman et al. 1997; Dachs et al. 1999). By these considerations, the contaminant inputs throughout food (zooplankton) and water will be reduced in summer (July) than in early spring (April). Beside this, during spring–summer fishes show their maximum growth and a consistent dilution of the contaminants already present into their body and of those coming from the new inputs is expected (Sijm et al. 1992). A similar pollution trend was observed by Binelli et al. (2004) in *Dreissena polymorpha*.

Statistical analyses reported in Tables 4 show no significant effect for sex (P>0.14). Moreover, lipid normalised organochloride concentrations were similar both in sperm and in eggs (differences <20%,) and between gonads and muscle (the maximum difference was of ~20% between gonad and muscle concentrations of total PCBs). Russell et al. (1999) described the maternal transfer of hydrophobic contaminants to eggs, showing that for oviparous organisms (specially fish) lipid-adjusted concentrations in eggs and maternal tissues were fairly close (lipid normalized egg-to-muscle tissue concentration ratio, EMR_L, equal to 1). EMR_L was independent of the fish's means of transferring lipid to eggs. Our data confirmed this thesis, and also that the approximate chemical equilibrium between contaminant concentrations in maternal tissues and eggs achieved in ovogenesis also occurs in spermogenesis. Moreover, the similarity of PCB and DDT composition in gonads (sperm and eggs) and fish muscles (Fig. 2) suggests the presence of a passive transport of lipid between a fish and its gonad. From a quantitative

Table 3 PCBs and DDTs concentrations in whitefish

Sample	Concentration ($ng\ g_{lip}^{-1}$)																							
	PCB congeners																			DDTs					
	95	101	118	132	138	149	151	153	170	174	177	180	183	187	194	195	201	203	206	*o,p′*-DDE	*p,p′*-DDE	*o,p′*-DDD	*p,p′*-DDD	*o,p′*-DDT	*p,p′*-DDT
M3-Apr	131	230	47	49	317	65	46	319	54	27	24	74	26	52	12	2.4	6.0	7.3	1.1	134	1,058	317	1,775	1.5	401
M4-Apr	137	190	58	123	471	80	58	416	63	36	35	88	36	73	16	4.3	11	11	1.1	298	1,460	458	3,040	3.7	780
F3-Apr	128	251	57	50	287	69	49	343	57	27	26	94	30	57	13	3.1	7.9	8.3	1.1	237	1,329	383	2,388	4.4	567
F4-Apr	199	383	77	76	565	95	71	578	86	40	38	150	43	57	24	4.3	13	14	1.6	253	2,262	325	4,677	2.5	1,110
M1-Jul	26	32	13	15	66	51	16	60	8	14	5.8	34	10	18	3.7	<0.5	3.7	5.9	<0.5	10	80	30	290	<1.0	50
M2-Jul	62	78	36	19	105	56	17	114	18	12	9.2	35	13	25	1.5	<0.5	2.3	3.4	3.3	55	769	87	1,051	<1.0	287
M3-Jul	76	137	38	41	219	52	34	216	50	27	21	75	23	41	10	1.8	7.0	9.5	0.5	96	795	201	1,335	2.2	349
F2-Jul	60	74	16	18	174	27	25	149	32	21	16	49	17	35	6.3	2.1	4.7	5.2	0.8	73	420	149	399	2.2	194
F3-Jul	79	80	64	24	160	96	34	149	36	21	17	38	18	37	7.5	2.2	5.6	6.7	0.5	14	793	207	960	3.3	241
F4-Jul	90	115	123	34	252	149	44	251	50	25	24	70	27	57	11	3.6	8.2	8.8	1.6	161	1,044	212	1,962	4.7	493
M1-Nov	112	148	46	42	255	64	50	280	55	28	26	73	28	59	14	2.8	8.2	9.4	<0.5	122	920	209	1,268	3.3	293
M2-Nov	71	108	83	33	216	134	40	186	42	24	21	53	21	43	8.6	3.0	5.5	6.2	0.7	59	1,303	124	1,732	1.2	314
M3-Nov	84	110	52	29	197	71	37	192	38	17	18	49	20	44	8.4	1.8	6.1	6.4	1.2	164	769	187	1,051	2.5	287
M4-Nov	85	140	92	45	305	104	39	274	51	24	25	79	28	59	11	3.5	8.8	9.1	<0.5	155	1,237	172	2,123	3.6	552
F1-Nov	65	82	30	24	156	42	29	140	31	17	13	38	16	34	6.6	1.8	4.8	5.3	0.6	70	470	100	570	<1.0	80
F2-Nov	75	114	126	64	259	174	29	202	41	18	20	55	20	43	8.5	2.6	6.9	6.2	0.6	96	722	135	676	<1.0	165
F3-Nov	88	125	50	38	212	67	37	196	35	22	23	64	26	50	9.4	2.6	7.0	7.8	1.1	145	918	161	1,390	4.4	346
F4-Nov	112	113	43	36	198	59	32	217	44	18	17	66	20	48	8.0	<0.5	6.7	7.9	<0.5	178	929	161	1,856	<1.0	342
F5-Nov	117	178	166	61	374	208	54	407	65	28	35	107	42	82	18	6.3	15	11	1.8	158	1,405	181	2,080	3.2	443
F6-Nov	124	208	108	68	448	109	59	455	94	35	38	153	53	105	25	6.8	15	19	2.3	246	1,571	173	2,758	6.6	641
Sperm	111	56	42	44	267	58	44	233	22	22	22	78	33	56	5.1	1.3	11	11	<0.5	106	2,480	135	3,864	<1.0	509
Eggs	93	47	31	40	233	43	33	213	27	20	13	53	20	40	6.7	3.1	13	6.7	<0.5	100	2,786	121	4,710	<1.0	647

Table 4 Statistical analysis (GLM) of the PCBs congeners concentration on lipid basis for sex and season as factors and age as a covariate

Compound	Statistical significance (*P*)			
	Cov: age *df*=1	Factor 1: sex *df*=1	Factor 2: season *df*=2	Interaction *df*=2
op'-DDE	0.0013**	0.81	0.0023**	0.75
pp'-DDE	0.00046***	0.51	0.0056**	0.053
op'-DDD	0.061	0.90	0.00002***	0.12
pp'-DDD	0.00024***	0.60	0.0018**	0.057
op'-DDT	0.65	0.37	0.48	0.52
pp'-DDT	0.00017***	0.71	0.0019**	0.0053**
ΣDDTs	0.00012***	0.57	0.00073***	0.046*
95	0.0091**	0.21	0.0003***	0.40
101	0.0069**	0.14	0.00005***	0.032*
118	0.026*	0.45	0.35	0.84
132	0.050	0.34	0.011*	0.45
138	0.0024**	0.82	0.0047**	0.81
149	0.14	0.62	0.47	0.91
151	0.0053**	0.54	0.0055**	0.23
153	0.00080***	0.48	0.0021**	0.40
170	0.0013**	0.51	0.049*	0.52
174	0.019*	0.88	0.039*	0.54
177	0.0015**	0.75	0.028*	0.59
180	0.00044***	0.35	0.057	0.13
183	0.00041***	0.63	0.10	0.74
187	0.00032***	0.88	0.079	0.79
194	0.0042**	0.47	0.068	0.59
195	0.00016***	0.21	0.068	0.88
201	0.00065***	0.54	0.18	0.80
203	0.0015**	0.87	0.53	0.59
206	0.12	0.94	0.62	0.56
ΣPCBs	0.0007***	0.46	0.0046**	0.84

*$P<0.05$; **$P<0.01$; ***$P<0.001$

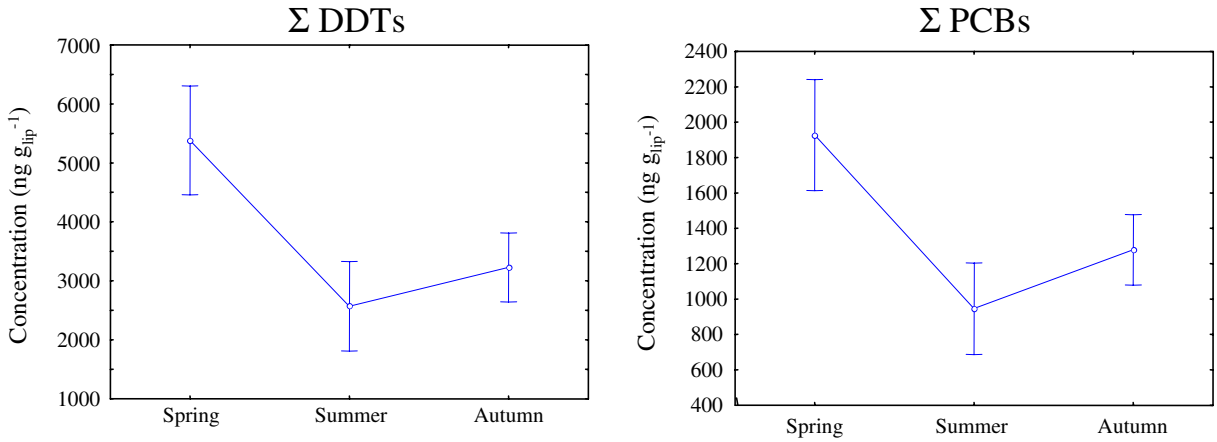

Fig. 1 Seasonal variation of the PCBs and DDTs concentrations in *C. macrophthalmus*. Means are adjusted for age as covariate and vertical bars denote 0.95 confidence intervals

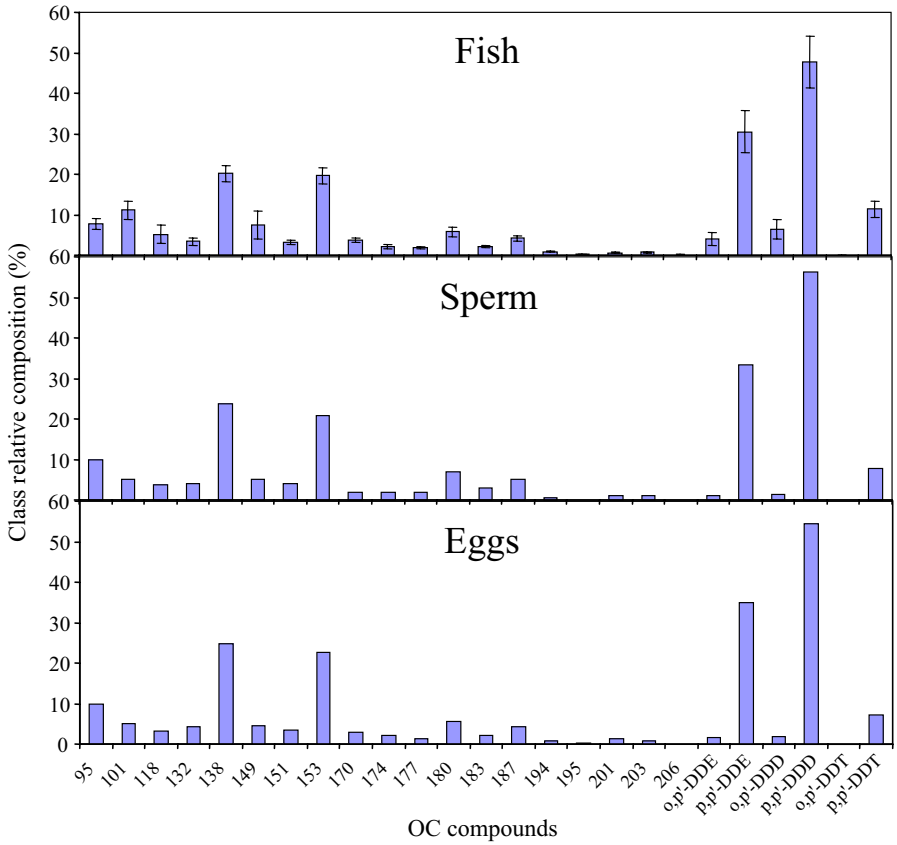

Fig. 2 Relative composition of the considered OC compounds in fish tissues and gonads (sperm and eggs) of *C. macrophthalmus*

point of view, the spawning process causes a net transfer of contaminants from adult females and males to eggs and sperm, respectively. As different amounts of the two gonad products are produced, a quantitative difference is expected in the overall transfer between female and male depending on the lipid content in eggs and sperm and on their masses. In whitefish the gonad mass, evaluated by the Gonadosomatic Index-GSI (percentage ratio between the weight of gonad and that of fish), was 3.4%±1.4 (standard deviation) and 13%±3.5 (standard deviation) for male and female, respectively (Giussani G., unpublished data). As the lipid content is 2.9% and 4.4% in sperm and eggs, respectively (Table 1), the contaminant burden transferred by females during the spawning process should be 5.8 times that of males. Nevertheless, any significant difference in pollutant levels was recognizable between males and females in muscle tissues in April (first sampling period after spawning). Two reasons can account for this. The contaminant amount transferred by female during reproduction is near 6 time that of male, but the total amount transferred in relation to the body burden is proportional to the GSI for female (13%). This means a contaminant depletion in female limited to a small percentage in relation to the body burden, and, for this reason, a concentration differences after spawning between the two sexes limited as well. The second reason deals with the behaviour of this species after reproduction and the elapsed time between spawning and our sampling season (April). In this species males remain on the spawning grounds more than females (Giussani, unpub. data). Energy is provided by perivisceral fat which is not completely utilized for the sperm building in summer–autumn. On the contrary, females which utilize completely perivisceral fat for the eggs maturation, need to eat actively after spawning. As in late winter–early spring water contamination is the highest (Bettinetti et al. 2006), the uptake of contaminants through diet should be also higher for females than for males, which are still on the spawning grounds and do not eat.

'Age' effect on contaminant levels in whitefish was found to be highly significant for almost all the

compounds examined (Table 4). Mean concentrations (adjusted for season as a covariate) revealed a similar and clear increasing trend with age both at a class and single compound level (Fig. 3). The 'age' effect has been reported as a typical characteristic of highly hydrophobic persistent compounds in fish (Schindler et al. 1995). Two factors are mainly involved in the increase of concentrations with age (Thomann and Connolly 1984). The first depends on the input from food: increasing the fish size will increase the amount and the size of its prey (Volta 2005), and therefore will increase the contaminant input to the gastrointestinal tract and therefore the concentration increase in the body tissues by the same mechanism which explains biomagnification (gastrointestinal magnification as described by Gobas et al. 1988, 1993). The second element related to the increase of concentrations with age is growth rate, which decreases as age increases. In the oldest fishes, as the growth dilution gets lower, contamination gets high.

3.3 Age-Dependent Model

The combination of the above mentioned elements gives the possibility to model the age-dependent bioaccumulation process, as proposed by Thomann and Connolly (1984). The concentration increases of total PCBs and total DDTs in whitefish is two-fold for an age interval between 1 and 6 years, much lower than that predicted by the model of Thomann and

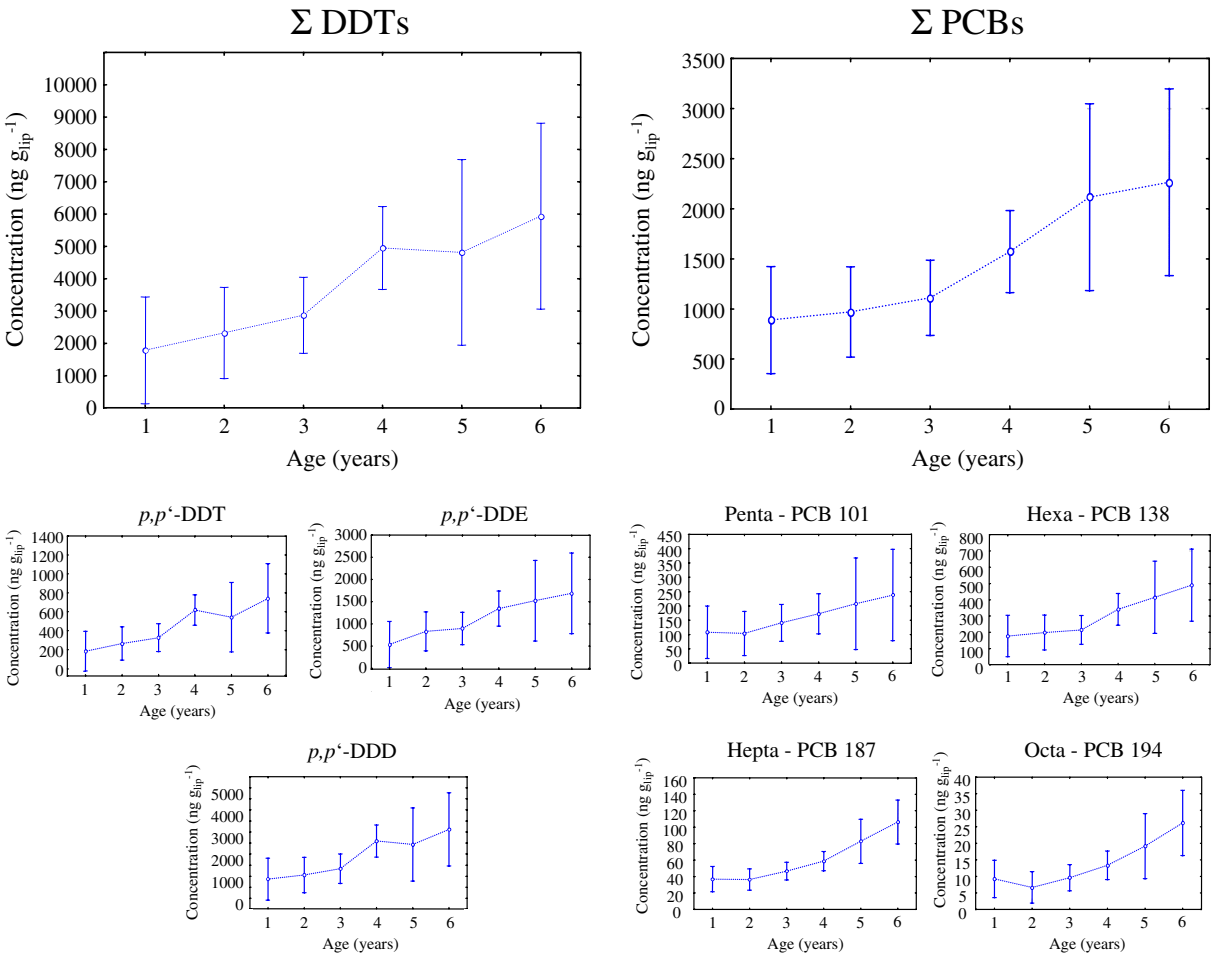

Fig. 3 Effect of age on the total PCBs and DDTs concentrations in *C. macrophthalmus* and on the concentrations of several single compounds (*p,p'*-isomers of DDTs and four PCB congeners coming from penta, hexa, hepta and octa chlorination group). Means are adjusted for season as covariate and vertical bars denote 0.95 confidence intervals

Connolly (1984), set up for lake trout. On the other hand, trout have a longer lifespan than whitefish, and change diet with size, from zooplankton to pelagic fish, while whitefish are always zooplanktophagous. Therefore, we developed an new model application (see Materials and Methods section) derived from the approach of Campfens and Mackay (1997). However, the basic condition for applying this model is the assumption of the steady-state at least in the time span of the fish life (1997–2005). Apart from small seasonal fluctuations, temporal trends in Lake Maggiore did not reveal any evident reduction in PCB contamination from 1997 to 2005 (Binelli et al. 2004; CIPAIS 2005), while a much greater variability was observed for DDTs, especially in their relative composition (Binelli et al. 2004; CIPAIS 2005). For this reason, only PCB were chosen for testing the model described in the Material and Method section. By the model structure, the age-dependent bioaccumulation process is expected to occur differently, depending on the fish characteristics, the K_{ow} of the compounds and the biotransformation process in the animal. Without biotransformation, the age-dependent bioaccumulation, as well as biomagnification, are maximal, and for each species dependent only on K_{ow}. Below a Log K_{ow} of 5, biomagnification does not significantly affect bioaccumulation (Thomann 1989; Fisk et al. 1998), but between a Log K_{ow} of 5 and 7, biomagnification can account for increases in concentrations of more than 1 order of magnitude (Thomann 1989; Fisk et al. 1998). Therefore, age-dependent bioaccumulation is expected to occur at the same K_{ow} interval and to depend on it. In this way, applying the developed model, an age-dependent bioaccumulation potential based only on K_{ow} (without biotransformation) can be calculated in whitefish for each compound, obtaining a value that can be defined as the 'maximum age-dependent bioaccumulation potential' or 'maximum age-dependent bioaccumulation factor' for that compound. It is called 'maximum' because it is calculated assuming no biotransformation, and it is called bioaccumulation factor because is dimensionless, being the ratio between the lipid-normalized concentration in fish of 6 year and that in fish of 1 year (C_{f6}/C_{f1}). Comparing these calculated maximum age-dependent bioaccumulation factor (without biotrasformation) with the experimental ones (derived from the experimental concentrations measured in field), which include biotransformation phenomena, the bioreactivity of each individual compound can be derived. As explained in Section 2 and following the approach propose by Arnot et al (2008), the difference between the K_{ow}-based age-dependent bioaccumulation potential without biotransformation (maximum value) and the experimental one, under steady state conditions, can be view as a measure of the biotransformation susceptibility of a compound in a given organism.

Before evaluating the biotransformation rate of the different congeners, the goodness of the model application used was tested on congeners 138 and 153, chosen for their very low susceptibility to metabolism. In Fig. 4 calculated (without biotransformation) and experimental relative increases for congeners 138 and 153 as functions of fish age are shown. The model of Campfens and Mackay (1997) predicts almost exactly the extent of the experimental concentration increase of the two congeners. Experimental and predicted relative increases from age class first to the sixth were almost identical for congeners 138 (2.8 and 2.7 respectively) and very close for congeners 153 (2.7 and 2.5 respectively). Relatively greater discrepancies were noticed for the intermediate age classes. For these small differences, uncertainties regarding the season-adjusted mean concentrations can be considered. Nevertheless, the results shown in Fig. 4 are fairly good and sufficiently accurate to conclude that the model is able to predict the age-dependent bioaccumulation process in whitefish. In Table 5 the physico-chemical properties and the relative increase of the experimental lipid-normalized concentrations between year 1 and 6 are reported for each congener, together with the predicted relative increase without biotransformation (maximum age-dependent bioaccumulation potential). For most of the congeners of Table 5, the predicted relative increases (without biotransformation) from the age class 1 to 6 were very close to the experimental ones, confirming that the metabolic transformation of such highly chlorinated PCBs by this fish is generally poor. The mean difference between predicted (without biotransformation) and experimental values was very low (14%). These considerations support the conclusion that for these pollutants, biotransformation processes in whitefish are very limited, according to Buckman et al. (2004). Plotting the experimental and predicted (without biotransformation) relative increase values versus Log K_{ow} data (Fig. 5), an obvious relationship

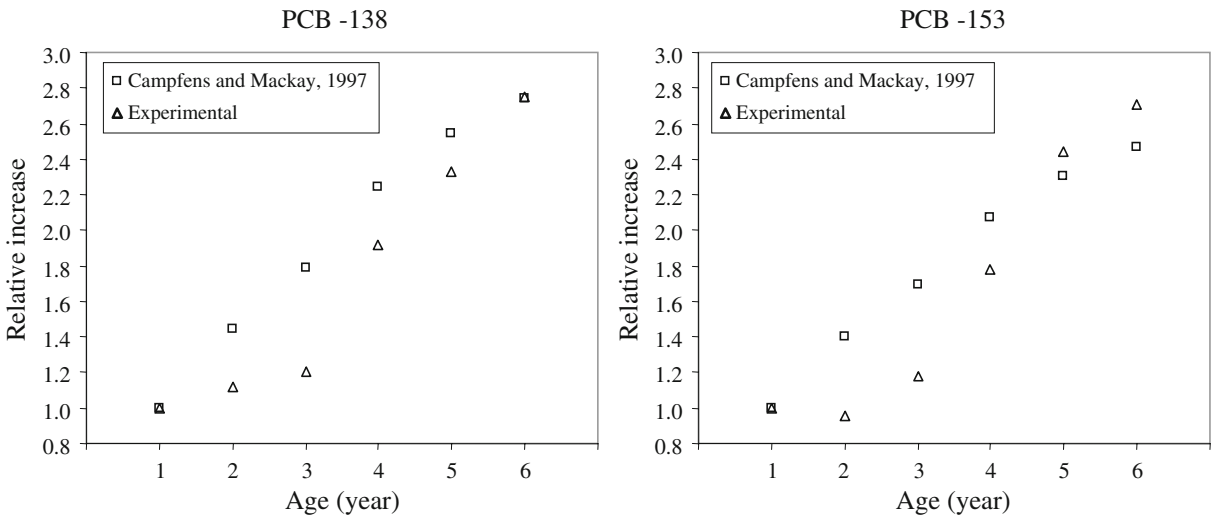

Fig. 4 Measured and predicted relative increase of the concentration of the congeners 138 and 153 in *C. macrophthalmus* in function to the fish age

is found for predicted values but also the experimental ones seems to suggest an increasing bioaccumulation potential related to K_{ow}. The linear regression was statistically significant (R^2=0.35; n=19; P=0.008). Even if several congeners seem to outstand the predicted trend (both above than below the predicted trend), the relationship between the experimental age-dependent bioaccumulation and the K_{ow} values confirms, once more, the low biotransformation susceptibility of these congeners in whitefish and the discussed analogies within the biomagnification process. The major discrepancies between predicted and measured data are evidenced in Fig. 5 with the indication of the congener number. Basing on our data, we interpret

Table 5 Physico-chemical properties (MW = molecular weight; K_{ow} = *n*-octanol water partition coefficient; H = Henry's constant) for the PCB congeners, experimental (Exp.) and modeled (Mod.) relative increase from the first age class to the sixth in whitefish

PCB congener	MW (g mol^{-1})	K_{ow}	H (Pa m^3 mol^{-1})	Exp. increase	Mod. increase
95	326.4	1.35×10^{6}[a]	30.6[b]	1.8	1.9
101	326.4	2.16×10^{6}[c]	24.1[c]	2.2	2.0
118	326.4	4.87×10^{6}[c]	14.5[c]	3.5	2.3
132	360.9	3.80×10^{6}[c]	20.5[c]	2.7	2.2
138	360.9	1.64×10^{7}[c]	30.1[c]	2.8	2.7
149	360.9	4.68×10^{6}[a]	24.0[b]	2.0	2.3
151	360.9	4.37×10^{6}[a]	28.7[c]	1.9	2.3
153	360.9	7.44×10^{6}[c]	19.8[c]	2.7	2.5
170	395.3	1.86×10^{7}[a]	8.85[b]	2.9	2.8
174	395.3	1.29×10^{7}[a]	17.13[b]	1.8	2.7
177	395.3	1.20×10^{7}[a]	16.5[b]	2.5	2.6
180	395.3	1.45×10^{7}[c]	8.13[c]	3.0	2.7
183	395.3	1.58×10^{7}[a]	20.4[b]	2.9	2.7
187	395.3	1.48×10^{7}[a]	20.5[b]	2.9	2.7
194	429.8	5.78×10^{7}[c]	4.40[c]	2.8	3.5
195	429.8	3.63×10^{7}[a]	12.0[b]	2.8	3.2
201	429.8	4.17×10^{7}[a]	13.2[b]	2.5	3.2
203	429.8	4.47×10^{7}[a]	14.2[b]	2.7	3.3
206	464.2	1.23×10^{8}[a]	8.8	3.9[d]	4.1

[a] Hawker and Connell (1988)

[b] Dunnivant et al. (1992)

[c] Li et al. (2003)

[d] This value was calculated considering the concentration below the limit of quantification equal to it.

them as mostly derived from the variability in the field-measured data (see Fig. 3) than an indication of an actual biotransformation capability. For this reason we do not try to calculate the biotransformation rate constant for the congeners which had an experimental age-dependent bioaccumulation factor lower than that calculated without biotransformation, even if it was mathematically possible with a simple rearrangement of equation 4.

3.4 Comparison of OC's Patterns in Fish and Fish-Eating Bird

Great crested grebe does not exclusively feed on whitefish, but on the most available pelagic fish of the lake (i.e. Piersma et al. 1988 Martinoli et al. 2003; Gagliardi et al. 2007). Anyway, the pelagic fish biomass in Lake Maggiore is constituted primarily by whitefish, which is, therefore, a very probable prey of great crested grebe.

The relative OC composition differences in fish and bird eggs of Lake Maggiore are shown in Fig. 6. The percentage differences (Fig. 6), obtained subtracting to the mean percentage of each congener in bird eggs the corresponding value in fish, were statistically checked by *t*-test. Only congeners 138, 180 and 183 resulted not significantly different. The disappearance of congener 149 in the great crested grebe eggs collected in Lake Maggiore was the most outstanding finding. In birds the biotransformation of PCB-149 seems to be complete, resulting in no residue transfer to eggs. The same congener was metabolized in the common cormorant (*Phalacrocorax carbo*) (Guruge and Tanabe 1997) and a metabolite of PCB-149, a methylsulfonyl-substitute, was found in birds (de Voogt et al. 1996). While PCB-149 has a *meta–para* unsubstituted position in one ring, congener 118 is hindered to those positions: probably this is the reason why the relative proportion of congener 118 in great crested grebe rose to above 15% from 5% abundance in whitefish. The finding that birds are not able to metabolize congeners 118 and 105 was previously noted by Borlakoglu et al. (1988) and Duinker et al. (1988). Also congeners 95, 101, 132, 151 and 174 were found in lower proportions in the bird species than in fish. Despite their difference in chlorination degree (from five to seven chlorine atoms), these congeners have a *meta–para* free-chlorine position. In particular congener 101 seems to be highly selectively metabolized by *P. cristatus* (relative composition change from 12% to 2%). Congeners 153 and 138 were also predominant in bird eggs collected in different part of the world, together with congeners 180, 110 and 118 (Focardi et al. 1988; Scharenberg 1991; Mora 1996; Guruge and Tanabe 1997; Kunisue et al. 2003).

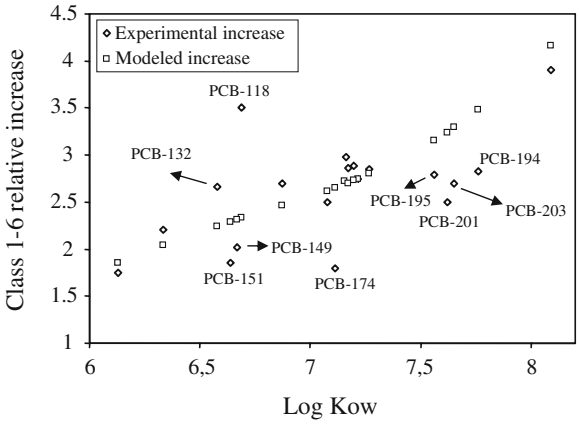

Fig. 5 Relationship between the measured and predicted (without biotransformation) relative increase and K_{ow} values for the single PCB congeners

Looking at DDT compounds (Fig. 6), great differences are evident in the bird's eggs pattern in comparison to the one for fishes in Lake Maggiore. Fish show a high capability to retain and bioaccumulate almost all DDT compounds. Birds, to the contrary, show a very efficient biotransformation of *pp'*-DDT, *p,p'*-DDD and their *o,p'* isomers, with a selective high bioaccumulation of *p,p'*-DDE. Very slow decreasing trends were observed for *p,p'*-DDE over a 1971–2001 time interval (16% for year) by Jörundsdóttir et al. (2006). The same authors found in guillemot (*Uria aalge*) eggs $MeSO_2$-derivatives of DDTs and PCBs that are even more persistent than the parent compound and highly accumulable as well because of their lipophilicity (only slightly lower than the parent compound) and because of their ability to bind specifically to proteins. Going up the food chain, the probability of finding modified patterns in comparison to the original mixtures, becomes greater, not only for the capability of the organism itself but also for that of the food chain that supports it, and so the necessity to look for possible metabolites that may be persistent and toxic becomes greater as well.

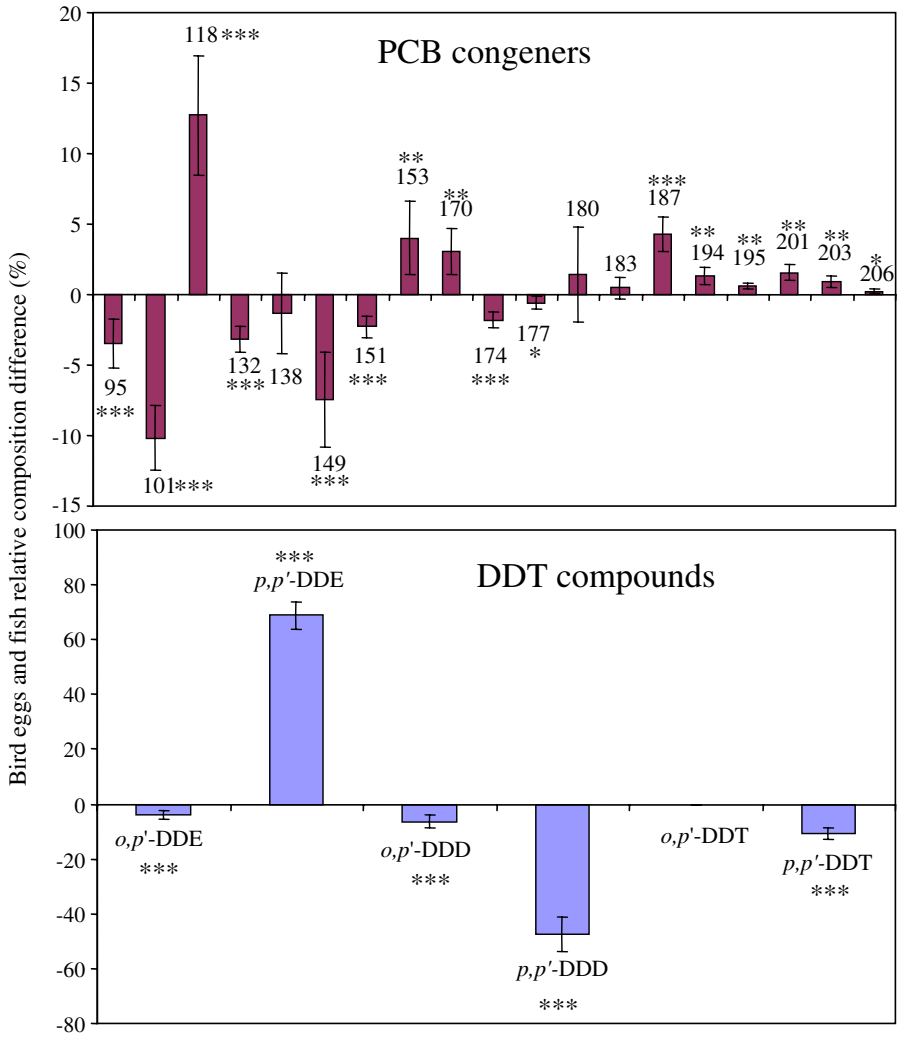

Fig. 6 Differences in the relative composition of the analysed PCBs and DDTs compounds in fish and bird eggs from Lake Maggiore. Differences were calculated as the relative composition in bird eggs minus that of fish. Data in bird eggs are described in CIPAIS (2005)

4 Conclusions

The case study of PCB and DDT pollution in Lake Maggiore permits several observations about bioaccumulation. Sex differences in extent of bioaccumulation are minor in whitefish, while season and fish age are important for both DDTs and PCBs. Overall differences in PCB and DDT concentrations in whitefish vary by about four fold as a result of these seasonal and age differences. These features must be carefully taken into account in the planning phase of monitoring studies and in the evaluation of exposure levels for humans and wild life. The observed seasonal trend has been shown to reflect the eco-physiological cycle of the fish and the contamination trends occurring in the water column during the year. The increase of the lipid-normalized concentrations with the increase of the fish age was interpreted by the same fugacity model explaining biomagnification. Two mechanism are involved: the increase of the contaminant input in the gastrointestinal tract for fish of increasing size, and the decrease of the dilution process for fish of increasing age. The increase in concentration from the age class 1 to class 6 was predicted quite well, and the low biotransformation susceptibility of such highly chlorinated congeners in whitefish was indirectly derived for almost all the analysed congeners. On the other hand, evidence of selective biotransformation was observed in a fish-eating bird by comparing the composition patterns of its eggs with that of fish from the same environment. Birds confirm themselves as highly metabolically

active organisms, able to substantially change DDT homologue and PCB congener profiles. A complete *p*, *p'*-DDT and *p*,*p'*-DDD metabolisation occurred as well as a complete biotransformation of congener 149, and a partial reduction of congeners 95, 101, 132, 151 and 174. Biotransformation is a mechanism of detoxification but also a source of new compounds eventually toxic for the organism itself and for their predators. Therefore, much more attention should be directed to identifying possible metabolites, such as $MeSO_2$-derivatives, for their possible persistence and toxicity to top predators.

Acknowledgements We would like to thanks Dr. Suzanne Levine for help with the English.

This work was partially supported by the financial contribution of the CIPAIS (Commissione Internazionale per la Protezione delle Acque Italo-Svizzere) and of the Province of Verbano Cusio Ossola (VCO), Italy

References

Arnot, J. A., Mackay, D., & Bonnell, M. (2008). Estimating metabolic biotransformation rates in fish from laboratory data. *Environmental Toxicology and Chemistry*, *27*, 341–351. doi:10.1897/07-310R.1.

Axelman, J., Broman, D., & Näf, C. (1997). Field measurements of PCB partitioning between water and planktonic organisms: Influence of growth, particle size, and solute-solvent interactions. *Environmental Science & Technology*, *31*, 665–669. doi:10.1021/es960088+.

ATDSR.(2000). *Toxicological profile for DDT, DDE and DDD*. US Department of Health and Human Services, Public Health Services, Syracuse.

Baker, J. E., Eisenreich, S. J., & Eadie, B. J. (1991). Sediment trap fluxes and benthic recycling of organic carbon, polycyclic aromatic hydrocarbons, and polychlorobiphenyl congeners in Lake Superior. *Environmental Science & Technology*, *25*, 500–509. doi:10.1021/es00015a019.

Berg, A., & Grimaldi, E. (1965). La biologia delle due forme di coregone (*Coregonus* sp.) del Lago Maggiore. *Memorie dell'Istituto Italiano di Idrobiologia*, *21*, 37–87.

Bettinetti, R., Croce, V., Galassi, S., & Volta, P. (2006). *p,p'*-DDT and *p,p'*-DDE accumulation in a food chain of Lake Maggiore (Northern Italy): Testing steady-state condition. *Environmental Science & Pollution Research*, *13*, 1–8. doi:10.1065/espr2006.01.001.

Binelli, A., Riccardi, F., & Provini, A. (2004). Present status of POP contamination in Lake Maggiore (Italy). *Chemosphere*, *57*, 27–34. doi:10.1016/j.chemosphere.2004.05.006.

Boon, J. P., Eijgenraam, F., Everaarts, J. M., & Duinker, J. C. (1989). A structure-activity relationship (SAR) approach towards metabolism of PCBs in marine animals from different trophic levels. *Marine Environmental Research*, *27*, 159–176. doi:10.1016/0141-1136(89)90022-6.

Borlakoglu, J. T., Wilkins, J. P. G., & Walker, C. H. (1988). Polychlorinated biphenyls in fish-eating seabirds—molecular features and metabolic interpretations. *Marine Environmental Research*, *24*, 15–19. doi:10.1016/0141-1136(88)90246-2.

Breivik, K., Sweetman, A., Pacyna, J. M., & Jones, K. C. (2002). Towards a global historical emission inventory for selected PCB congeners—A mass balance approach. 1. Global production and consumption. *The Science of the Total Environment*, *290*, 181–198. doi:10.1016/S0048-9697(01)01075-0.

Buckman, A. H., Brown, S. B., Hoekstra, P. F., Solomon, K. R., & Fisk, A. T. (2004). Toxicokinetics of three polychlorinated biphenyl technical mixtures in rainbow trout (*Oncorhynchus mykiss*). *Environmental Science & Technology*, *23*, 1725–1736.

Bush, B., Tumasonis, C. F., & Backer, F. D. (1974). Toxicity and persistence of PCB homologs and isomers in the avian system. *Archives of Environmental Contamination and Toxicology*, *2*, 195–212. doi:10.1007/BF01956169.

Calamari, D., Tremolada, P., Di Guardo, A., & Vighi, M. (1994). Chlorinated hydrocarbons in pine needles in Europe: Fingerprint for the past and recent use. *Environmental Science & Technology*, *28*, 429–434. doi:10.1021/es00052a014.

Campfens, J., & Mackay, D. (1997). Fugacity-based model of PCB bioaccumulation in complex aquatic food webs. *Environmental Science & Technology*, *32*, 577–583. doi:10.1021/es960478w.

CIPAIS.Commissione Internazionale per la Protezione delle Acque Italo-Svizzere, 2005. *Monitoraggio della presenza del DDT e di altri contaminanti nell'ecosistema Lago Maggiore*, Annual Report. Calderoni A. (Ed), CNR-ISE, Verbania (Italy).

Connolly, J. P., & Pedersen, C. J. (1988). A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. *Environmental Science & Technology*, *22*, 99–103. doi:10.1021/es00166a011.

Clark, K. E., Gobas, F. A. P. C., & Mackay, D. (1990). Model of organic chemical uptake and clearance by fish from food and water. *Environmental Science & Technology*, *24*, 1203–1213. doi:10.1021/es00078a008.

Dachs, J., Eisenreich, S. J., Baker, J. E., Ko, F. C., & Jeremiason, J. D. (1999). Coupling of phytoplankton uptake and air–water exchange of persistent organic pollutants. *Environmental Science & Technology*, *33*, 3653–3660. doi:10.1021/es990168o.

de Vault, D. S., Hesselberg, R., Rodgers, P. W., & Feist, T. J. (1996). Contaminant trends in lake trout and walleye from Laurentian Great Lakes. *Journal of Great Lakes Research*, *22*, 884–895.

de Voogt, P., van Raat, P., & Rozemeijer, M. (1996). Methyl sulfonyl PCBs in cormorant chicks from The Netherlands. *Organohalogen Compounds*, *28*, 517–521.

Debruyn, A. M. H., Ikonomou, M. G., & Gobas, F. A. P. C. (2004). Magnification and toxicity of PCBs, PCDDs, and PCDFs in upriver-migrating Pacific salmon. *Environmental Science & Technology*, *38*, 6217–6224. doi:10.1021/es049607w.

Drouillard, K. G., Fernie, K. J., Smits, J. E., Bortolotti, G. R., Bird, D. M., & Norstrom, R. J. (2001). Bioaccumulation and toxicokinetics of 42 polychlorinated biphenyl congeners in American kestrels (*Falco sparverius*). *Environmental Toxicology and Chemistry*, *20*, 2514–2555. doi:10.1897/1551-5028(2001)020<2514:BATOPB>2.0.CO;2.

Duinker, J. C., Schulz, D. E., & Petrick, G. (1988). Selection of chlorinated biphenyl congeners for analysis in environmental samples. *Marine Pollution Bulletin*, *19*, 19–25. doi:10.1016/0025-326X(88)90748-5.

Dunnivant, F. M., Elzerman, A. W., Jurs, P. C., & Hasan, M. N. (1992). Quantitative structure–property relationships for aqueous solubilities and Henry's law constants of polychlorinated biphenyls. *Environmental Science & Technology*, *26*, 1567–1573. doi:10.1021/es00032a012.

Fisk, A. T., Norstrom, R. J., Cymbalisty, C. D., & Muir, C. G. (1998). Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with the octanol/water partition coefficient. *Environmental Toxicology and Chemistry*, *17*, 951–961. doi:10.1897/1551-5028(1998)017<0951:DAADOH>2.3.CO;2.

Focardi, S., Leonzio, C., & Fossi, M. C. (1988). Variations of polychlorinated biphenyl congener composition in eggs of Mediterranean water birds in relation to their position in the food chain. *Environmental Pollution*, *52*, 243–255. doi:10.1016/0269-7491(88)90127-3.

Gagliardi, A., Martinoli, A., Preatoni, D., Wauters, L. A., & Tosi, G. (2007). From mass of body elements to fish biomass: a direct method to quantify food intake of fish eating birds. *Hydrobiologia*, *583*, 213–222. doi:10.1007/s10750-006-0528-y.

Giussani, G., & de Bernardi, R. (1977). Food selectivity of *Coregonus* sp. in Lago Maggiore: An energetical approach. *Memorie dell'Istituto Italiano di Idrobiologia*, *34*, 121–130.

Gobas, F. A. P. C., Muir, D. C. G., & Mackay, D. (1988). Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. *Chemosphere*, *17*, 943–962. doi:10.1016/0045-6535(88)90066-5.

Gobas, F. A. P. C., Zhang, X., & Wells, R. (1993). Gastrointestinal magnification: The mechanism of biomagnification and food chain accumulation of organic chemicals. *Environmental Science & Technology*, *27*, 2855–2863. doi:10.1021/es00049a028.

Guruge, K. S., & Tanabe, S. (1997). Congener specific accumulation and toxic assessment of polychlorinated biphenyls in common cormorants, *Phalacrocorax carbo*, from Lake Biwa, Japan. *Environmental Pollution*, *96*, 425–433. doi:10.1016/S0269-7491(97)00045-6.

Hawker, D. W., & Connell, D. W. (1988). Octanol–water partition coefficients of polychlorinated biphenyl congeners. *Environmental Science & Technology*, *22*, 382–387. doi:10.1021/es00169a004.

Henriksen, E. O., Gabrielsen, G. W., Trudeau, S., Wolkers, H., Sagerup, K., & Skaare, J. U. (2000). Organochlorines and possible biochemical effects in glaucous gulls (*Larus hyperboreus*) from Bjørnøya, the Barents Sea. *Archives of Environmental Contamination and Toxicology*, *38*, 234–243. doi:10.1007/s002449910031.

Jeremiason, J. D., Hornbuckle, K. C., & Eisenreich, S. J. (1994). PCBs in Lake Superior, 1978–1992: Decreases in water concentrations reflect loss by volatilisation. *Environmental Science & Technology*, *28*, 903–914. doi:10.1021/es00054a023.

Jörundsdóttir, H., Norström, K., Olsson, M., Pham-Tuan, H., Hühnerfuss, H., Bignert, A., et al. (2006). Temporal trend of bis (4-chlorophenyl) sulphone, methylsulfonyl-DDE and PCBs in Baltic guillemot (*Uria aalge*) egg 1971–2001—A comparison to 4,4'-DDE and PCB trends. *Environmental Pollution*, *141*, 226–237. doi:10.1016/j.envpol.2005.08.054.

Kunisue, T., Watanabe, M., Subramanian, A., Titenko, A. M., & Tanabe, S. (2003). Congener-specific patterns and toxic assessment of polychlorinated biphenyls in resident and migratory birds from southern India and Lake Baikal in Russia. *Archives of Environmental Contamination and Toxicology*, *45*, 547–561. doi:10.1007/s00244-003-2213-8.

Li, N., Wania, F., Lei, Y. D., & Daly, G. L. (2003). A comprehensive and critical compilation, evaluation, and selection of physical–chemical property data for selected polychlorinated biphenyls. *Journal of Physical and Chemical Reference Data*, *32*, 1545–1590. doi:10.1063/1.1562632.

Mackay, D. (2001). *Multimedia Environmental Model. The Fugacity Model,* 2nd ed. Lewis: Boca Raton.

Martinoli, A., Gagliardi, A., Preatoni, D. G., Di Martino, S., Wauters, L. A., & Tosi, G. (2003). The extent of Great Crested Grebe predation on Bleak in Lake Como, Italy. *Waterbirds*, *26*, 201–208. doi:10.1675/1524-4695(2003)026[0201:TEOGCG]2.0.CO;2.

Marvin, C. H., Murray, C. N., Stern, G. A., Braekevelt, E., Reiner, E. J., & Painter, S. (2003). Spatial and temporal trends in sediment contamination in Lake Ontario. *Journal of Great Lakes Research*, *29*, 317–331.

Matthews, H. B., & Andersen, M. W. (1975). Effect of chlorination on the distribution and excretion of polychlorinated biphenyls. *Drug Metabolism and Disposition: the Biological Fate of Chemicals*, *3*, 371–380.

Mora, M. A. (1996). Congener-specific polychlorinated biphenyl patterns in eggs of aquatic birds from the lower Laguna Madre, Texas. *Environmental Toxicology and Chemistry*, *15*, 1003–1010. doi:10.1897/1551-5028(1996)015<1003:CSPBPI>2.3.CO;2.

Nakata, H., Tanabe, S., Tatsukawa, R., Amano, M., Miyazaki, N., & Petrov, E. A. (1995). Persistent organochlorine residues and their accumulation kinetics in Baikal seal (Phoca sibirica) from Lake Baikal, Russia. *Environmental Science & Technology*, *29*, 2877–2885. doi:10.1021/es00011a026.

Paterson, G., Drouillard, K. G., & Haffner, D. (2007). PCB elimination by yellow perch (Perca flavescens) during an annual cycle. *Environmental Science & Technology*, *41*, 824–829. doi:10.1021/es060266r.

Piersma, T., Lindeboom, R., & van de Eerden, M. R. (1988). Foraging rhythm of great crested grebes *Podiceps cristatus* adjusted to diel variations in the vertical distribution of their prey *Osmerus eperlanus* in a shallow eutrophic lake in The Netherlands. *Oecologia*, *76*, 481–486.

Renaud, C. B., Comba, M. E., & Kaiser, L. E. (1999). Temporal trend of organochlorine contaminant levels in the North-Eastern part of Lake Superior basin based on lamprey larvae lipid burdens. *Journal of Great Lakes Research*, *25*, 918–929.

Rogan, W. A., & Chen, A. (2005). Health risks and benefits of bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT). *Lancet*, *366*, 763–773. doi:10.1016/S0140-6736(05)67182-6.

Russell, R. W., Gobas, F. A. P. C., & Haffner, G. D. (1999). Maternal transfer and in ovo exposure of organochlorines in oviparous organisms: a model and field verification. *Environmental Science & Technology*, *33*, 416–420. doi:10.1021/es9800737.

Scharenberg, W. (1991). Cormorants (*Phalacrocorax carbo sinensis*) as bioindicators for polychlorinated biphenyls. *Archives of Environmental Contamination and Toxicology*, *21*, 536–540. doi:10.1007/BF01183875.

Schindler, D. W., Kidd, K. A., Muir, D. C. G., & Lockhart, W. L. (1995). The effects of ecosystem characteristics on contaminant distribution in northern freshwater lakes. *The Science of the Total Environment*, *160/161*, 1–17. doi:10.1016/0048-9697(95)04340-7.

Sijm, D. T. H. M., Seinen, W., & Opperhuizen, A. (1992). Life-cycle biomagnification study in fish. *Environmental Science & Technology*, *26*, 2162–2174. doi:10.1021/es00035a014.

Solcà, N., De Rossa, M., & Jermini, M. (2006). Monitoring of DDTs in fishes of Lake Maggiore. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene*, *97*, 348–357.

Tanabe, S., Watanabe, H., Kan, F., & Tatsukawa, R. (1988). Capacity and mode of PCB metabolism in small cetaceans. *Marine Mammal Science*, *4*, 103–124. doi:10.1111/j.1748-7692.1988.tb00191.x.

Thomann, R. V. (1989). Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environmental Science & Technology*, *23*, 699–707. doi:10.1021/es00064a008.

Thomann, R. V., & Connolly, J. P. (1984). Model of PCB in the Lake Michigan trout food chain. *Environmental Science & Technology*, *18*, 65–71. doi:10.1021/es00120a003.

UNEP (2001). *Stockholm convention on persistent organic pollutants*. UNEP: Geneva.

Van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunstrom, B., Cook, P., Feeley, M., et al. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental Health Perspectives*, *106*, 775–792. doi:10.2307/3434121.

Volta, P. (2005). *Contaminazione da DDT nelle acque del Lago Maggiore e trasferimento nella fauna ittica pelagica*. PhD dissertation. Italy: University of Milan.

Walker, C. H. (1990). Persistent pollutants in fish-eating birds bioaccumulation, metabolism and effects. *Aquatic Toxicology (Amsterdam, Netherlands)*, *17*, 293–324. doi:10.1016/0166-445X(90)90014-G.

Wyss, P. A., Mühlebach, S., & Bickel, M. H. (1982). Pharmacokinetics of 2,2', 4,4', 5,5' -hexachlorobiphenyl (6-CB) in rats with decreasing adipose tissue mass. 1. Effects of restricting food intake two weeks after administration of 6-CB. *Drug Metabolism and Disposition: the Biological Fate of Chemicals*, *10*, 657–661.