Research Articles

pp'DDT and pp'DDE Accumulation in a Food Chain of Lake Maggiore (Northern Italy): Testing Steady-State Condition

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In memory of Davide Calamari

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Abstract

Background, Aims and Scope. Although pp'DDT usage was strongly limited or banned in most parts of the world during the last three or four decades, the parent compound, its homologues and their metabolites still occur at levels which might pose a risk for many ecosystem components.
A case of DDT pollution of industrial origin was discovered in 1996 in Lake Maggiore, the second largest (212 km^2) and deepest (370 m) lake in Italy, causing concern for wildlife and human health.
The extensive monitoring of many biotic and abiotic compartments which followed from 1998 in order to assess the pollution level and its trend in time, provided a great availability of data referring to DDT contamination of the different fish species of the lake.
In this study, the recent contamination levels in selected fish species were compared to those measured in 1998 to evaluate the temporal pollution trend of the lake and its natural recovery, given that no remediation measures were carried out on the contaminated soils and sediments in this time span. Moreover, a modelling approach to test the equilibrium condition between water and pelagic fish species was used. Analytical results of pp'DDT and pp'DDE concentrations in lake water were used as input data in the bioenergetic model by Connolly & Pedersen (1988) to calculate concentrations in two fish species and to compare the predicted and the measured contamination.

Methods. Sampling and analytical determination of DDT homologues in lake water: Five water sampling campaigns were carried out from May 2002 to February 2004 in three sampling sites of Lake Maggiore. Suspended and dissolved pollutants were determined separately. Quantitative DDT homologue analyses were performed by HRGC coupled with ECD detection by the external standard method. Single water extracts were put together in correspondence with the stratification zones of the water column inferred on the basis of the temperature profile to improve analytical sensitivity.
Selection of fish data: Concentrations of DDT and DDE in fishes were selected from recent literature (CIPAIS 2003, 2004).
Bioaccumulation model: The bioenergetic model proposed by Connolly & Pedersen (1988) was used to assess the bioaccumulation of pp'DDT and pp'DDE of *Alosa fallax* (landlocked shad) and *Coregonus* spp. (whitefish), selected among the different species as representative of a secondary consumer level.

Results and Discussion. The average concentrations of pp'DDT and pp'DDE in water to be used as input data in the bioenergetic model were obtained considering all the concentrations measured at the three sampling stations in the epylimnion where the fish species considered in this study spend most of their life. The resulting values were 0.05 and 0.16 ng/L for pp'DDT and pp'DDE, respectively.

Average measured pp'DDT and pp'DDE concentrations in landlocked shad were 0.81 ± 0.39 and 1.69 ± 0.71 mg/kg lipids, respectively, and were 0.29 ± 0.12 and 1.06 ± 0.41 mg/kg lipids for the whitefish. Calculated and measured values turned out to be in quite good agreement for pp'DDT, while measured pp'DDE concentrations were higher than expected on the basis of the bioenergetic model in both species. Probably metabolic transformations of pp'DDT accumulated in fish tissues in the past are responsible for the observed differences between calculated and expected pp'DDE concentrations in fish.

Conclusions. Pelagic fishes of Lake Maggiore seem to maintain the DDT accumulated during their life time and the most efficient mechanism responsible for the fish population recoveries is probably their generation changes; for this reason, equilibrium models cannot be used until negligible pp'DDT concentrations are reached in fish tissues.

Recommendations and Outlook. The limit proposed for pp'DDT in water by the EU Directive 2000/60, which will come in force in 2008, is 0.2 ng/L, four times higher than the average concentration measured in Lake Maggiore waters. Nevertheless, concentrations measured in Lake Maggiore fish were very close and sometimes exceeded the Maximum residue limits (MRLs) settled by the Italian legislation for foods (0.1 mg/kg w.w. for fish containing 5–20% lipid). It seems, therefore, that the 'environmental quality standard' of 0.2 ng/L cannot guarantee the suitability of fish for human consumption.

Keywords: Bioenergetic model; DDE, DDT concentrations in fish; fish; food chain; Lake Maggiore; trophic chains

Introduction

Although pp'DDT usage has been strongly limited or banned in most parts of the world during the last three or four decades, the parent compound, its homologues and their metabolites still occur at levels causing concern to wildlife and human health in many areas of the planet. This is mainly due to:

1. DDT production and usage against malaria and other tropical diseases, as well as illegal uses (UNEP 2001, Tren and Bate 2004);
2. production for further transformation into Dicofol, an acaricide still in use (van de Plassche et al. 2002);
3. long range transport and concentration in cold areas (Galassi et al. 1997, Blais et al. 1998, Wania 1999, Blais et al. 2001);
4. abandoned industrial areas where DDT was produced in the past and landfill where it was disposed.

Industrial DDT contamination is still a problem of great concern, since sites where pp'DDT was produced in the past, like the Montrose Torrance manufacture in California and the Velsicol Chemical Corp. (formerly Michigan Chemical Corp.) in Michigan (USEPA 1999, USEPA 2005), whose DDT productions were stopped more than 30 years ago, still represent local sources of pollution; in fact the half-life of the parent DDT in soil is 2–15 years (USEPA 1989) and the persistence of its main metabolite, pp'DDE is even higher (Boul 1994). Also sediments can act as repositories for DDT and its metabolites, serving as sources for these compounds for long periods of time, given the long half-lives of these compounds and their resistance to biodegradation (Sanger et al. 1999). Moreover, some DDT plants are still operating around the world (van de Plassche et al. 2002), such as a pesticide manufacturer in Spain probably responsible for the contamination of the basin of the River Cinca, a tributary of the River Ebro. The only Italian manufacturer of pp'DDT working until 1996 was located in the Lake Maggiore basin, the second largest Italian subalpine lake (212 km^2). Eight years after the closing of the DDT plant ordered by the Ministry of the Environment, when control laboratories found that DDT concentration in fishes exceeded the allowed threshold for human consumption, the lake ecosystem is still polluted by the parent compound and by its main metabolites, at such an extent to cause concern for aquatic life, wildlife species and human health (Bettinetti et al. 2005). Obviously, contamination levels in fish are subjected to fluctuations due to their physiological modifications and to the changes of the hydrological conditions of the tributaries and of the lake itself; nevertheless, it should be expected that the interruption of DDT synthesis will lead to a gradual recovery of the lake.

As a matter of fact, several data referring to DDT-like compounds are available for different fish species of Lake Maggiore (CIPAIS 1999, 2003, 2004), but a univocal temporal trend is not immediately recognizable both because of seasonal variability and very likely because of the heterogeneity of the fish size. A careful selection of the rough data, therefore, is needed before using them to evaluate the contamination trend of the lake ecosystem and to verify the equilibrium condition in the lake ecosystem by predictive food web models.

However, the modelling approach to assess the risk for DDT accumulation in top predator species of Lake Maggiore implies the knowledge of the pollutant concentrations in water which could not be determined in previous studies (CIPAIS 1999), being always below the detection limit of 1–2 ng/L.

To overcome this problem, zebra mussel (*Dreissena polymorpha*) was used as a sentinel species in a previous study (Galassi and Cassi 2001), assuming that biomagnification capacity for pp'DDT and pp'DDE was negligible in this organism, which should be considered, therefore, as a good indicator of bioconcentration from water only. Nevertheless, zebra mussel is a filter-feeding organism living attached to rocks of the littoral zone, where high fluctuations of pollutant concentrations are expected to occur in correspondence with the increase of the river discharges and with other temporary phenomena which cause sediment re-suspension and solid transport from the basin. For these reasons, zebra mussel might not be representative for the pelagic environment, where the pollutant concentrations should be more constant than in the littoral zone, strongly influenced by the river inputs and lake currents.

So, in order to verify the suitability of predictive steady-state models to forecast the evolution of Lake Maggiore, further attempts were done to determine DDT homologue concentrations in the water column of the lake. With this aim, five water sampling campaigns were organized from May 2002 to February 2004 in three sampling sites of Lake Maggiore, representative of the pelagic environment (**Fig. 1**). Samples, taken at different depths from the surface to the bottom of the lake, were analysed individually and then put together on the basis of the thermal stratification of the lake in order to improve the analytical sensitivity.

Analytical results of pp'DDT and pp'DDE average concentrations in the lake water were used as input data in a bioenergetic model (Connolly and Pedersen 1988) to test the equilibrium condition with some fish species (CIPAIS 2003, 2004). For doing so, data variability in fish analyses was minimized by selecting samples of rather homogeneous size within the species and species whose concentration variabilities during the sampling period resulted in lower than average pollutant concentrations.

The present contamination level in the selected fish species was then compared to that measured in 1998 to evaluate the temporal pollution trend of the lake and its natural recovery, since no remediation measures have been carried out on the contaminated soil and sediments in this time span.

1 Materials and Methods

1.1 Sampling and analytical determination of DDT homologues in lake water

Water samples were collected with a Van Dorn bottle in June and October 2002, in May and November 2003 and in March 2004 at 0, 5, 10, 20, 50, 100, 150, 200, 250, 300, 350 m at the deepest point of the lake (Ghiffa, site no. 1), at 0, 5, 10, 20, 50, 100, 150 m at Pallanza sampling station (site no. 2) and at 0, 5, 10, 20, 50, 100 m at Lesa sampling station (site no. 3) (see Fig. 1), probably influenced by DDT inputs through the River Toce. Samples were transported in dark glass bottles and filtered within 24 hours. After filtration on 0.45 µm cellulose acetate filters, 2 litres of water sample were added with 100 ml of *n*-hexane (Merck, pesticide analysis grade) and stored at 4°C until extraction, that was carried out by 1 h magnetic stirring directly in the bottle used to store the sample in order to minimize DDT homologues loss for volatilization or adsorption onto the glass walls. Extraction was repeated with 75 and 25 ml of *n*-hexane, respectively, stirring the two phases for 15 min. Hexane extracts were combined, reduced to a small volume under vacuum and cleaned up on a Florisil® column (40 mm x 0.7 mm I.D.) eluting with 25 ml of *n*-hexane.

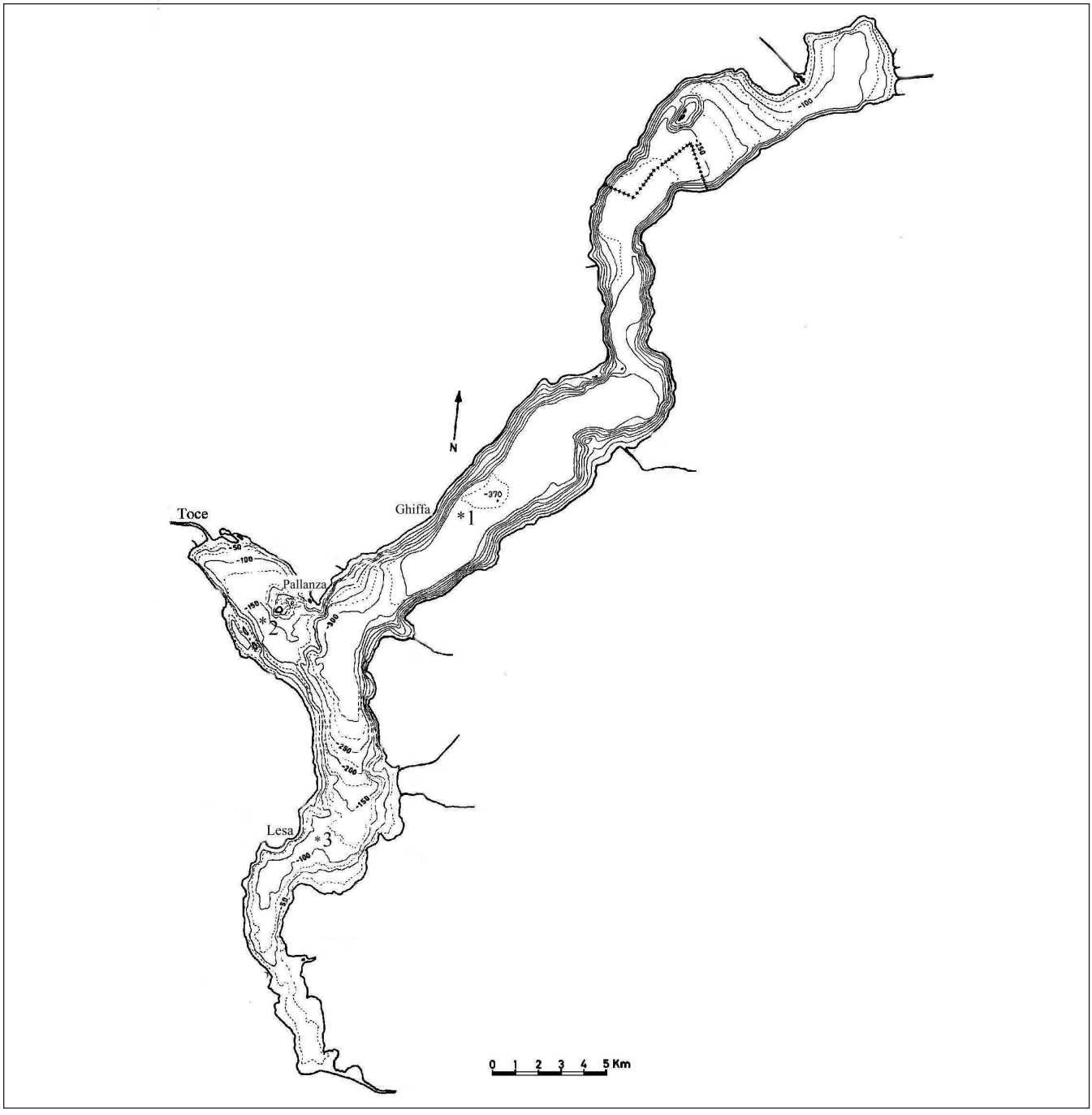

Fig. 1: Map of Lake Maggiore. The sampling stations are indicated by an asterisk with a number (Ghiffa=1, Pallanza=2, Lesa=3)

Purified water extracts were concentrated to 0.5 ml and analysed by gas-chromatography (Fison Top 8000) coupled to electron capture detection under the following conditions:

- Injection system: On-column (volume injected: 1 µl)
- Column: CP-Sil-8 CB, 50 m x 0.25 mm I.D., film thickness 1.2–2 µm
- Temperature programme: from 60 to 180°C at 20°C min^{-1}, followed by a run from 180 to 250 at 3°C min^{-1} and a further run from 250 to 270 at 2°C min^{-1}
- Carrier gas: Helium at 0.7 ml min^{-1}
- Auxiliary gas: Nitrogen at 30 ml min^{-1}

Integrated samples, obtained by putting together the single depth extracts in agreement of the hepy- and hypolimnion layers inferred from the temperature profile, were further concentrated and analysed under the same conditions.

Quantitative DDT homologue analyses were performed by the external standard method using a solution containing pp'DDT, pp'DDE, pp'DDD, op'DDT, op'DDE, op'DDD, prepared from single pure compounds (Alltech) in *n*-hexane, as a reference standard.

The detection limit for single sample analysis was 0.05 ng/L for each DDT homologue.

Then, single extracts were put together in correspondence with the stratification zones of the water column and concentrated again to exactly 0.5 ml. In this way, detection limits as low as 0.01 ng/L could be achieved.

1.2 Selection of fish data

pp'DDT and pp'DDE concentrations measured in six fish species which are typical inhabitants of the pelagic and the littoral of the lake, were carefully selected among those reported by CIPAIS (2003, 2004), considering the pool samples composed by at least six fishes of both sexes of rather homogeneous body size. Based on average body length and weight, only adult fishes were selected for all the species. Data referring to fishes sampled in July and September 2002, in July and December 2003 and in April 2004 were considered in correspondence with pp'DDT and pp'DDE water concentrations measured in this work.

1.3 Bioaccumulation model

The bioenergetic model proposed by Connolly and Pedersen (1988) assesses the bioaccumulation of organic compounds by aquatic organisms both from water and through the food chain. According to the model, the bioaccumulation factor (BAF_L) expressed on a lipid basis increases through the food chain in relation to the *n*-octanol/water coefficient (K_{ow}) of the compound and to the trophic level of the organism.

This tendency for a pollutant to be transported through the trophic chain is called 'fugacity'; a fugacity ratio (η) can be calculated from each trophic level to the next level below, if the K_{ow} value of the chemical and some physiological parameters of the species linked by a trophic relationship are known. In this study, the fugacity ratios for *Alosa fallax lacustris* Fatio, 1890 (landlocked shad) and *Coregonus* spp. (whitefish), selected among the different species as representative of a secondary consumer level, were calculated according to the equations reported by Connolly and Pedersen (1988), adjusted to the actual average lipid content and weight of the two fish species considered in this study.

2 Results and Discussion

2.1 Water analyses

Only pp'DDE could quantitatively determined in single 2L samples, while pp'DDT and pp'DDD concentrations where very often below the detection limits (0.05 ng/L). Using integrated samples, the detection limit could be lowered to 0.01 ng/L and both pp'DDT and pp'DDE concentrations could be determined in all the epylimnetic samples of the deepest station (Ghiffa). The intermediate metabolite pp'DDD was very frequently undetectable, even in integrated samples, and it was not considered for modelling purposes.

Samples from Pallanza and Lesa stations could not be analysed in the first campaign because there were many interfering peaks in the gas-chromatograms, very likely due to organic matter carried into the lake by the Toce River flood which occurred in May 2002.

A sharp increase of pp'DDT and pp'DDE concentrations was observed in November 2003 at Ghiffa station, both in the epylimnion and in the hypolimnion (**Table 1**). Since this event is quite anomalous and episodic, the average concentrations of pp'DDT and pp'DDE in water to be used as input data in the bioenergetic model was calculated as the median value, obtained considering all the concentrations measured at the three sampling stations in the epylimnion. In fact, most of the fish species considered in this study spend most of their life in this layer (Berg and Grimaldi 1966).

The resulting values were 0.05 and 0.16 ng/L for pp'DDT and pp'DDE, respectively.

2.2 pp'DDT and pp'DDE concentration in fishes

Fish data normalized for the lipid content are presented in **Table 2.** The term whitefish (*Coregonus* spp.) comprises both the pollan ('lavarello', *Coregonus* sp. hybrid form deriving from *C. wartmanni coeruleus* Fatio, 1890 and *C. schinzii helveticus* Fatio, 1890) and the houting ('bondella', *Coregonus macrophthalmus* Nüsslin, 1882) because in Lake

Table 1: pp'DDT and pp'DDE concentrations in the epylmnion and hypolimnion layers from June 2002 to March 2004 in three different sites of Lake Maggiore (see Fig. 1). In March 2004, only one value is reported because the lake has circulated

			pp'DDT (ng/L)	pp'DDE (ng/L)		pp'DDT (ng/L)	pp'DDE (ng/L)		pp'DDT (ng/L)	pp'DDE (ng/L)
		Layers (m)	Ghiffa		Layers (m)	Pallanza		Layers (m)	Lesa	
June '02	Epylimnion	0–50	0.04	0.18		n.d.	n.d.		n.d.	n.d.
	Hypolimnion	100–350	<0.01	<0.01		n.d.	n.d.		n.d.	n.d.
October '02	Epylimnion	0–50	0.03	0.16	0–30	0.05	0.25	0–30	0.01	0.14
	Hypolimnion	100–350	0.01	0.21	50–150	0.02	0.11	50–100	0.01	0.03
May '03	Epylimnion	0–100	0.05	0.22	0–30	0.09	0.23	0–30	0.02	0.16
	Hypolimnion	150–350	0.02	0.14	50–150	<0.01	0.33	50–100	<0.01	0.19
November '03	Epylimnion	0–50	0.82	0.86	0–30	0.02	0.11	0–30	0.07	0.11
	Hypolimnion	100–350	0.55	0.62	50–150	0.16	0.09	50–100	<0.01	0.05
March '04			0.07	0.06		0.10	0.18		0.05	0.08

n.d. = not determined

Table 2 Average concentrations and standard deviation (s.d.) of pp'DDT and pp'DDE in Lake Maggiore fishes from July 2002 to April 2004 (data recalculated from CIPAIS 2003, 2004)

		Landlocked	Whitefish	Perch	Chub	Rudd	Tench
pp'DDT (mg/kg lipids)	July '02	1.23	0.29	0.06	0.06	0.13	0.19
	September '02	0.45	0.15	0.04	0.04	0.10	0.10
	July '03	0.75	0.45	0.45	0.63	0.13	0.46
	Dec '03	0.63	0.20	0.17	0.05	0.12	0.08
	April '04	1.01	0.45	0.78	0.15	0.14	0.50
	Average	**0.81**	**0.31**	**0.30**	**0.19**	**0.12**	**0.27**
	s.d.	**0.31**	**0.14**	**0.31**	**0.25**	**0.02**	**0.20**
pp'DDE (mg/kg lipids)	July '02	1.36	0.57	0.45	0.48	0.76	5.77
	September '02	1.86	1.22	0.23	0.56	0.87	1.65
	July '03	1.15	1.14	1.91	2.13	8.67	3.50
	Dec '03	1.22	0.75	0.97	0.68	1.04	0.33
	April '04	2.87	1.62	4.22	1.15	1.93	1.00
	Average	**1.69**	**1.06**	**1.56**	**1.00**	**2.65**	**2.45**
	s.d.	**0.71**	**0.41**	**1.62**	**0.68**	**3.39**	**2.20**

Maggiore the two species, introduced in the Lake at the end of 800 and in 1950, respectively, with the aim of increasing the fishery, can be easily distinguishable only according to their maximum size (Grimaldi and Manzoni 1990).

Two of the six fish species of Table 2 resulted quite stabily concerning pp'DDT and pp'DDE concentrations during the two-year period considered in the present study: the landlocked shad and the whitefish. These two species, whose standard deviation was less than 45.2% of their respective average concentration, were selected as being representative of the second level fish community and their contaminant concentrations were compared with the values obtained by the bio-energetic model by Connolly and Pedersen (1988).

Moreover, it has to be said that the concentrations of DDT measured in all fish species exceed the thresholds for the human consumption of the Italian law set at 0.05 and 0.1 mg/kg w.w. of total DDT for fishes with a lipid content of > 5% and within the range of 5–20%, respectively, groups in which all of the species reported in Table 1 are comprised (CIPAIS 2003, 2004).

Concentrations in pelagic fish species. According to the bioenergetic model by Connolly and Pedersen (1988) the equation for calculating the bioaccumulation of zooplanktivorous fishes is:

$$Cf_L = K_{ow} * C_w * \eta_3 \quad \text{(Eq. 1)}$$

where:

Cf_L: concentration of the chemical lipid-normalized in fish

C_w: measured concentration of the chemical in water

K_{ow}: *n*-octanol/water partition coefficient of the chemical, being 501187 for pp'DDE and 1548816 for DDT (Mackay et al. 1997)

$$\eta_3 \text{ (fugacity ratio)} = 1 + f_3 + f_3 * f_2 \quad \text{(Eq. 2)}$$

f_3 and f_2, referred to fish and zooplankton, respectively, were calculated using **Eq. 3**:

$$f = \frac{\alpha C}{GR + \frac{1000 W^{-0.2}/FL}{K_{ow}}} \quad \text{(Eq. 3)}$$

where the average weight (W), the average lipid content (L), the average growth rate (GR), the consumption rate (C) and the efficiency of food assimilation (α) of the two selected fish species and of zooplankton are those reported in **Table 3** (Connolly and Pedersen 1988). W and L of fishes were calculated as the average value of those reported for the two considered species (CIPAIS 2003, 2004).

Table 3: Average weight (W) and lipid content (L), growth rate (GR), consumption rate (C) and efficiency of food assimilation (a) of zooplankton and of two selected fish species considered in the bioenergetic model (Connolly and Pedersen 1988, CIPAIS 2003, 2004)

Organisms	W (g)	L	GR (1/day)	C (g/g day)	α
Zooplankton	0.001	0.03	0.04	0.30	0.6
Landlocked shad	139.70	0.073	0.0035	0.03	0.6
Whitefish	231.15	0.034	0.0035	0.03	0.6

Using the Eq. 3, *f* values for zooplankton can be calculated as follows:

- for DDE

$$f_{2DDE} = \frac{0.3*0.6}{0.04 + \frac{1000*0.001^{-0.2}}{501187}/0.03} = \mathbf{0.59}$$

- and for DDT

$$f_{2DDT} = \frac{0.3*0.6}{0.04 + \frac{1000*0.001^{-0.2}}{1548816}/0.03} = \mathbf{1.43}$$

while *f* for landlocked shad is:

- for DDE

$$f_{3landlockedshad}DDE = \frac{0.03*0.6}{0.0035 + \frac{1000*139.7^{-0.2}}{501187}/0.073} = \mathbf{1.32}$$

- and for DDT

$$f_{3landlockedshad}DDT = \frac{0.03*0.6}{0.0035 + \frac{1000*139.7^{-0.2}}{1548816}/0.073} = \mathbf{2.65}$$

For the whitefish, the following *f* values can be calculated:

- for DDE

$$f_{3whitefish}DDE = \frac{0.03*0.6}{0.0035 + \frac{1000*231.15^{-0.2}}{501187}/0.034} = \mathbf{0.77}$$

- and for DDT

$$f_{3whitefish}DDT = \frac{0.03*0.6}{0.0035 + \frac{1000*231.15^{-0.2}}{1548816}/0.034} = \mathbf{1.82}$$

The fugacity ratios (η_3) of landlocked shad and of the whitefish of Lake Maggiore were calculated using Eq. 2:

$\eta_{3\ (landlocked\ shad\ DDE)} = 1 + 1.32 + 1.32 * 0.59 = 3.10$
$\eta_{3\ (landlocked\ shad\ DDT)} = 1 + 2.65 + 2.65 * 1.43 = 7.44$
$\eta_{3\ (whitefish\ DDE)} = 1 + 0.77 + 0.77 * 0.59 = 2.22$
$\eta_{3\ (whitefish\ DDT)} = 1 + 1.82 + 1.82 * 1.43 = 5.42$

Then the theoretical concentration (Cf_L) of pp'DDT and pp'DDE for both fish species were calculated using Eq. 1:

for landlocked shad Cf is:

Cf_{LDDE} = 501187 * 0.16 ng/L * 3.10 = **0.25** mg/kg lipids

Cf_{LDDT} = 1548816 * 0.05 ng/L * 7.44 = **0.58** mg/kg lipids

and for the whitefish is:

Cf_{LDDE} = 501187 * 0.16 ng/L * 2.22 = **0.18** mg/kg lipids

Cf_{LDDT} = 1548816 * 0.05 ng/L * 5.42 = **0.42** mg/kg lipids

In **Table 4**, the theoretical concentrations (Cf_L) are then compared with the average concentrations of pp'DDT and pp'DDE measured in fish from July '02 to April '04 (CIPAIS 2003, 2004).

Table 4: Average calculated (Cf_L) and measured (M) (± s.d.) pp'DDT and pp'DDE concentrations in landlocked shad and whitefish (CIPAIS 2003, 2004)

	pp'DDT (mg/kg lipids)		pp'DDE (mg/kg lipids)	
	Cf_L	M	Cf_L	M
Landlocked shad	0.58	0.81 (±0.31)	0.25	1.69 (±0.71)
Whitefish	0.42	0.31 (±0.14)	0.18	1.06 (±0.41)

In both fishes, calculated and measured values fit better for pp'DDT than for pp'DDE.

Given that higher levels of pp'DDT should occur in the oldest fish specimen, the measured pp'DDE concentration is much higher than expected on the basis of the bioaccumulation model. A possible explanation could be found supposing that the oldest fishes maintain the memory of the past contamination that should be more conservative for pp'DDE than for its parent compound. Pp'DDE body burden, exceeding the concentration deriving from bioconcentration and biomagnification, should be the result of the metabolic transformation of pp'DDT accumulated in past years. In facts, dechlorination of pp'DDT to pp'DDE was observed in fishes in laboratory experiments by the induction of the mixed-function oxidase (MFO) in the liver (Schmitt et al. 1985, Wang and Simpson 1996). If we consider two different pp'DDE accumulation pathways in fish muscles, a two compartment model (Clark et al. 1987), which takes into account the exchanges between the abiotic environment and the fish blood, which is a fast process, and between the blood and the fats, which is much slower, should be more appropriate in this case (Arnot and Gobas 2004), based on the condition that the metabolic rate of the dechlorination of pp'DDT to pp'DDE is known. Very likely an equilibrium condition will not be reached between the abiotic compartments and the biota until pp'DDT concentra-

Table 5: Measured (M) and calculated (C) Log BAF for pp'DDE and pp'DDT in three different pelagic fish species of Lake Ontario (data by Oliver and Niimi 1988)

	pp'DDE		pp'DDT	
	M	C	M	C
Small smelt	6.374	5.224	5.835	5.674
Large smelt	6.534	5.423	6.334	5.908
Alewife	6.374	5.541	6.265	5.947

tion in tissues becomes negligible in comparison to that of the more stable metabolite pp'DDE.

This hypothesis is strengthened by a study performed in Lake Ontario in the earlier '80 (Oliver and Niimi 1988), when DDT contamination in fishes was comparable to that recently found in Lake Maggiore. In that work, DDT homologues and PCB concentrations were analytically determined in water and in several fish species. The BAFs (bioaccumulation factors) for fishes were then calculated dividing the pollutant concentrations in fish (as wet weight) by those determined in water, obtaining linear relationships between log BAF and Log K_{ow} values for each species. However, when the experimental equations reported for the pelagic fishes alewife (*Alosa pseudoharengus*) and smelt (*Osmerus mordax*), which can be considered the 'ecological analogues' of those considered in the present work, are applied to calculate the LogBAF values for pp'DDT and pp'DDE (**Table 5**) discrepancies between calculated and measured pp'DDE concentrations are observed; in this case, as for the Lake Maggiore, the measured values resulted higher by one order of magnitude than the predicted ones as well.

Comparing pp'DDE and pp'DDT concentrations in fishes referred to the 2002–2004 period with those measured during 1998, no significant differences (*t-Student test* for independent samples) both for pp'DDT and pp'DDE in fish are observed (**Table 6**). However, the most polluted fishes collected in 1998 presented higher pp'DDT and pp'DDE levels than those measured during 2002–2004. The higher variability of the data of fishes collected in 1998 leads one to suppose that the oldest fishes (>3 years) sampled in 1998, living in the lake before the closure of the DDT manufacturer, should have stored high DDT levels in their tissues. As a matter of fact, a decreasing trend in concentrations has been observed from 1996 to 1998, but a sudden increase in concentration newly happened in 2001 after a flood in 2000 which transferred pollutants from the industrial site and resuspended the deeper contaminated sediments within the lake (CIPAIS 2003); this fact allows to conclude that, although the DDT manufacturing plant was closed six years ago, local pollution sources are still present in the lake watershed causing pulse contamination on the occasions of heavy rains periods followed by river floods.

3 Conclusions

The pelagic fishes of Lake Maggiore seem to be far from an equilibrium condition with the water column. Fishes maintain the most persistent pollutant, pp'DDE, accumulated during their life time and the most efficient mechanisms responsible of the lowering of the concentrations could be their generation changes: Probably the oldest fishes caught in 1998 still suffered from the discharges of the manufacturing plant which worked until 1996, while those fished in 2002–2004 suffered from the consequences of a flood event occurred in 2000.

Moreover, even if DDT homologue concentrations measured in water seem to provide a good indication for forecasting the transport of the chemicals through the pelagic food chain, more specific information should be obtained on the role of DDT metabolism in fish species. If this aspect is not clarified, only very rough predictions can be performed, whichever model is used to calculate the concentrations in fish species.

Finally, it has to be said that in the framework of the UE Directive 2000/60, aimed to prevent the aquatic environment from pollution by dangerous substances, 'quality standards' have been provided for organochlorine insecticides, being 0.2 ng/L for pp'DDE and pp'DDT and 0.3 ng/L for pp'DDD (D.L. 367/2003). Nevertheless, concentrations measured in fish from Lake Maggiore, where the average pp'DDT concentration in water was four times lower than the quality standard, were very close and sometimes exceeded the Maximum residue limits (MRLs) settled by the Italian legislation for foods (0.1 mg/kg w.w. for fish containing 5–20% of fat). Therefore, the quality standard of 0.2 ng/L for pp'DDT cannot guarantee the suitability of fish for human consumption. Moreover, it has to be considered that pp'DDE, which is the main metabolite found in fish-eating birds of Lake Maggiore (Galassi et al. 2002), acts as an anti-androgenic chemical. Negative effects on top predator populations could occur as a consequence of the secondary poisoning.

Table 6: Average lengths (± s.d.), pp'DDT and pp'DDE concentrations (± s.d.) measured in landlocked shad and whitefish of Lake Maggiore in 1998 and in the period 2002–2004 (data recalculated from CIPAIS 1999, 2003, 2004)

	1998			2002–2004		
	Mean length (cm)	pp'DDT (mg/kg lipids)	pp'DDE (mg/kg lipids)	Mean length (cm)	pp'DDT (mg/kg lipids)	pp'DDE (mg/kg lipids)
Landlocked shad	27.8 (±3.1)	2.37 (±1.93)	3.91 (±2.84)	23.7 (±3.4)	0.81 (±0.31)	1.69 (±0.71)
Whitefish	27.6 (±3.3)	0.45 (±0.33)	1.02 (±0.78)	27.4 (±5.7)	0.31 (±0.14)	1.06 (±0.41)

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