FOOD CHAIN ACCUMULATION OF DDT AND SOME OF ITS METABOLITES IN FISH FROM LAKE MAGGIORE (ITALY) AFTER AN ACCIDENTAL RELEASE - EVALUATED BY THE USE OF STABLE NITROGEN ISOTOPES AND OTHER BIOTIC PARAMETERS.

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Introduction

Lake Maggiore (or Verbano) is a lake situated in North-west Italy, on the borders of Piedmont , Lombardy and Switzerland, and is the second largest Italian lake, after Garda. It has an area of 212.2 sq. km. and lies at an altitude of 193 m; its deepest point is 370 m, with an average depth of 177 m. During routine monitoring of contamination in fish from Lake Maggiore in the summer 1996, Swiss authorities found DDT at levels well exceeding the average contamination measured in the last few years. The ratios between DDT and metabolites also indicated a recent contamination. The rise in concentration led the Italian authorities to prohibit fishing in Lake Maggiore in order to protect the population from consuming contaminated fish. From a biological and toxicological point of view, DDT and metabolites are well known for their capability of being bioaccumulated in the food chain, reaching high concentrations in the upper trophic levels.

These chemicals (in particular o,p-DDT but also p,p- DDT) have recently shown estrogenic activity which can cause impairing effects on wildlife (especially fish) reproduction, that can, in turn, lead to important ecological effects.

The reason for the increase in concentration were related to “recent” discharges of DDT coming from an industrial plant located on a Lake Maggiore tributary.

Since professional fishing of fish belonging to *Coregonus spp* (the most important species with about 84% of the total fish catch, with about 600 t/y) in Lake Maggiore. is economically important, a number of research activities were carried out in order to assess a possible effect on fish population (1,2).

In this work we used this data to evaluate possible accumulation and biomagnification of DDT and some of its metabolites in fishes of different ages and sizes. We sampled fish of different age classes belonging to *Coregonus spp.,* of the two “forms”, “lavarello “ and “bondella”, which feed mainly on zooplankton.

To evaluate possible biomagnification, due to variation in diet style, stable nitrogen isotopes were used and an simple model of exponential food chain accumulation are compared with possible lipid -water equilibrium partitioning.

Materials and methods

Thirty-three fishes of two forms of *Coregonus spp*. (bondella and lavarello) were sampled from two localities in the lake (Caldè and Cerro) using floating nets. For the DDT and metabolites studies about 20 g of dorsal muscles from each fish were cut out, freeze-dried, extracted in hexane/acetone (9:1) with Soxtec for 6 hours. The extracts were then digested with sulfuric acid (on Extrelut columns) for 4 hours in order to remove the lipid fraction. The samples were then eluted and purified on silica gel chromatography. The elution solvent was a mixture of hexane and toluene (65:35). The eluted fraction was concentrated in rotary evaporator and then evaporated with a gentle flux of nitrogen. Internal standards ($^{13}C_{12}$ p,p'-DDT and p,p'-DDE) were added at extraction time. The analysis was performed with GC-MS (HP 6890-5972a) in EI in selected ion monitoring mode. The column used was a SGE BP10, 50m (0.22mm i.d., 25 μm phase thickness). Lipid determinations were carried out by evaporating and weighting the organic phase extracted and the lipid content of the fishes varied between 0.9 and 4.3 % of fresh weight. Age of the fishes were determined by gill rakers analysis .and varied between 2 an 6 years. All fish were sampled in the winter season, between October and January.

For the isotopic analysis a subsample of the fish muscles was grinned and dried (60 ^{o}C). The samples were analysed for Delta (δ) ^{15}N with an element analyser/isotope ratio MS instrument having a precision of +- 0,1 $^{o}/_{oo}$. Isotopic compositions are reported relative to N_2 in air (Delta^{15}N). The Delta–values given N in the fish samples are calculated according the following general equation (here Delta (δ) ^{15}N):

$$\delta^{15}N = \frac{\left(\frac{^{15}N}{^{14}N}\right)_{sample} - \left(\frac{^{15}N}{^{14}N}\right)_{air}}{\left(\frac{^{15}N}{^{14}N}\right)_{air}} * 1{,}000$$

A more detailed description of the basis of calculation of δ^{15}N are given in (3) and (4).

Results and discussion

This whole concept of using stable isotops of nitrogen is based on the empirical findings that the heavy isotope is enriched in organisms, whereas the "common" isotope is not (5;6). The method has a potential to classify the trophic positions of the organisms in continuous variables instead of discrete variables as with the traditional method. The advantage of this method is that it describes the trophic interrelation of the organisms with hopefully greater realism and precision than the traditional method, which is based on estimations from food composition analysis. If there is an increase in fugacity of a compound during food digestion and/or hindered elimination capacity, in combination with a limited metabolic ability this will leads to organism concentrations which exceeds the possible organism lipid -water equilibrium partitioning. In such case it is theoretically possible to expect increasing concentrations with increasing trophic levels i.e. biomaginfication.

Determinations of trophic relationships using the Delta^{15}N-technique has been previously (op. cit.) used to evaluate the potential of biomagnification of different hydophobic organic compounds (HOCs). The concentration of a persistent HOCs in organisms can be calculated according to the following, if it is assumed that the substance is transferred with approximately the same efficiency between all different trophic levels:

$$[HOC] = A * e^{B*\delta^{15}N}$$

In the equation, A is the concentration of the substance at the base of the food chain and B is a measure of the biomagnification potential of the substance. The concentration increases from one trophic level to the next if B is positive (i.e. biomagnification). B-values of substances that are significantly metabolised or excreted are negative.
In this investigation op-, ppDDD, op-, ppDDE and op-, ppDDT were correlated to the Delta ^{15}N and fitted to the linear model stated above. The results showed that the relatively easily excreted (or more easily degraded) metabolites of DDT i.e. DDD showed poor correlations and/or negative B values. As an example of this type of compound which does not biomagnify is shown in Figure 1 in which ppDDD is plotted against Delta ^{15}N. The same poor correlation was also shown for the opDDD metabolite.

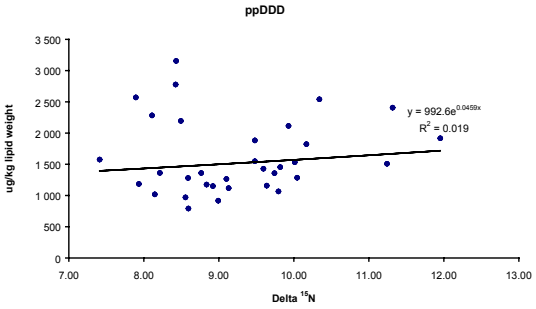

Figure 1 Regression between ppDDD (ug/kg lipid weight) and Delta ^{15}N

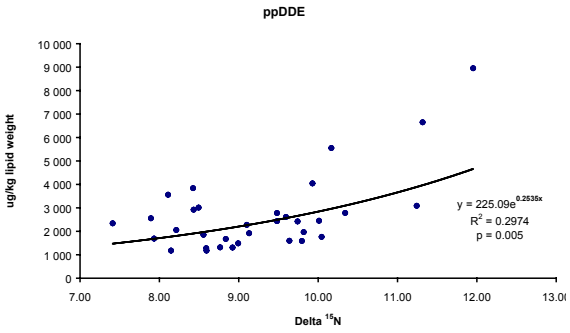

Figure 2 Regression between ppDDE (ug/kg lipid weight) and Delta ^{15}N

The more stabile metabolite DDE as well as DDT showed all a positive correlation with Delta ^{15}N indicating a biomagnification potential. As an example ppDDE is plotted against Delta ^{15}N in Figure 2. The B-value i.e. the "biomagnifying power" of this metabolite in the investigated food web were 0.25. This was also one of the highest B-values of the investigated compounds, only exceeded by ppDDT (B-value of 0.29) and followed by opDDT (B-value of 0.21) and opDDE (B-value of 0.20).
The correlation between Delta ^{15}N and age were significant ($p>0.001$) but with an R^2 of 0.50. However, there were no or very poor correlations between lipid based concentrations of the DDTs, and all its investigated metabolites, and age in the data set. This is probaly related to the fact that fish diet does not change dramatically as the age increases, since this species feeds mainly on zooplankton. The trend of total DDT concentrations in muscles is also shown in figure 3, where lipid corrected concentrations appear to be relatively constant for fishes up to age class 4. Fish of age class 5 instead present much higher concentrations which can only be related to higher concentrations in the food at the time, probably indicating the time period of the contamination.

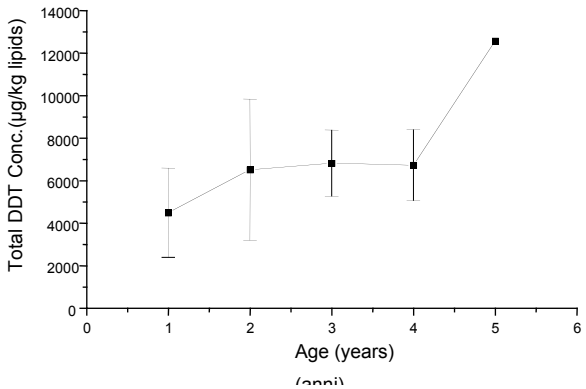

Figure 3 Total DDTs concentrations in muscles in different age classes of "lavarello".

The initial results reported allow to state that there are weak correlations between change in accumulation of DDTs in fish and age, indicating that the food composition does not change significantly, while the analysis of chemical concentrations reveals an increased accumulation of DDTs, probably at the time of the accident, most likely due to the more contaminated food.

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