Chemosphere 94 (2014) 190–198

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Organochlorine pesticide residues: An extensive monitoring of Italian fishery and aquaculture

Maurizio Masci *, Elena Orban, Teresina Nevigato

Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Research Centre for Food and Nutrition, Rome, Italy

HIGHLIGHTS

- The lipid composition of fish is unique among all living species.
- Fish consumption has relevant positive effects on human health.
- Residues of OC pesticides are detectable in the entire fish food chain.
- OC pesticides have adverse effects on human health included carcinogenesis.
- Fish analyzed is safe as regards OCPs especially when the lipid content is below 2%.

ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form 27 September 2013
Accepted 1 October 2013
Available online 1 November 2013

Keywords:
Organochlorine pesticides (OCPs)
Fishery
Aquaculture
Mass spectrometry
Analytical quality control
Persistent Organic Pollutants (POPs)

ABSTRACT

A sampling campaign from 21 sites in Italy was carried out to investigate the presence of organochlorine pesticide residues in different fish species. Samples came from marine fishery and either from sea- or freshwater aquaculture. Fish feed used in some fish farms were also analyzed. Pesticides studied belong to Persistent Organic Pollutants widely used in the past such as DDT, chlordane, heptachlor, and others. To ensure good quality results and proper data validation the main existing guidelines in the field were applied. The instrumental technique was a Dual column–Dual detector Gas Chromatography (GC–ECD and Ion Trap GC–MS) which allowed that complementary data on the same sample were acquired. Results for fishery showed a wide range of concentrations depending from the area and species examined. DDT, the major OC pesticide detected, varied from 0.02 to 130.03 ng g^{-1} edible portion. As regards the products of aquaculture we observed slightly lower average levels of pollutants in a more narrow range of concentration: this is probably due to fish feed used as shown by some measures performed in the present study. Organochlorine pesticide residues were detected in all samples examined but they were generally well below the existing tolerance or action levels. Also the estimated daily intakes are well below than those recommended by WHO. This is a good indication about OCPs in the areas investigated but some further considerations on fish safety must be taken into account. An example on how fishes may act as bioindicators is reported.

1. Introduction

Organochlorine pesticides (OCPs) were largely used in the past against pests, epidemics and unwanted vegetable species. The production of these chemicals in the twentieth century has been impressive: global production of aldrin and dieldrin was estimated to be 13 000 tons in 1972 (WHO, 1989), worldwide production of DDT in 1974 was 60 000 tons (WHO, 1979), worldwide production of pure HCB was estimated to be 10 000 tons/year for the years 1978–1981 (WHO, 1997). Along with their efficiency OCPs showed later their negative aspects such as persistency and toxicity to humans even at very low concentrations (long term effects due to chronic exposure). Not degradability and high solubility in organic matter allowed them to enter in the food chain as contaminants therefore they were banned for use in most western countries more than thirty years ago. Today OCPs are ubiquitous and still detectable in the entire fish food chain. For this reason organochlorine pesticides, together with other Chlorine-based compounds such as Dioxins and PCBs, were debated in Stockholm Convention on POPs, in 2001, where controlled disposal of organochlorines still available and global ban for use were proposed. The Convention entered in force in May, 2004. The Marine Ecosystem is particularly exposed to pollution being at the lowest altitude and so acting as an "end-point" of any type of pollutant created on land. A similar reasoning is valid for rivers and lakes

* Corresponding author. Address: Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Research Centre for Food and Nutrition, Via Ardeatina 546, 00178 Rome, Italy. Tel.: +39 0651494464; fax: +39 0651494550.
E-mail address: maurizio.masci@entecra.it (M. Masci).

0045-6535/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2013.10.016

that are at lower level than fields and hills around them. High lipid solubility and low water solubility lead to the retention of organochlorine pollutants in the fatty tissues of fish; subsequently organochlorine pesticides tend to migrate worldwide and can be detected in organisms remote in time and geographical area from the point of exposure. Fish consumption is reported to have beneficial effects on health mainly for the particular composition of lipids rich in omega-3 and in polyunsaturated fatty acids that are associated with a significantly lower risk of fatal and total coronary heart diseases (Whelton et al., 2004; Zhang et al., 2010). Other positive effects were observed towards a number of different pathologies (Moyad, 2005). On the other hand organochlorine pesticide residues are suspected to cause cancer in the consumer and to affect a wide range of organ systems including the liver, lungs, kidneys, thyroid, reproductive tissues and nervous and immune systems (WHO, 1979, 1997). It is important, therefore, that the level of these xenobiotics in fish is constantly monitored to be certain that it is below a reasonable level of risk. The environmental contamination by POPs may be entirely non-uniform, being possible that some points or restricted areas are highly contaminated in a context of a mid-low contamination. For this reason a broad monitoring it is required to have at least an idea about the level of these xenobiotics in the fish products of a region. In the present work 26 fish samples from Italian catching areas and 10 fish samples from Italian aquacultures were analyzed for OCP residues. Fish feed used for 7 of the 10 aquaculture fish samples were also investigated.

2. Materials and methods

2.1. Fish samples

Table 1 reports all fish samples analyzed in the present work.

As regards marine fishery a wide range of species was collected from the most known and consumed to the lesser known but important at the local level. A number of 15 species was investigated by executing a total of 26 samplings. Among these species common cuttlefish (*Sepia officinalis*) was included which although not a fish (mollusk) is, however, very important in the context of catching activity.

As regards fish farming (either from sea- or freshwater aquaculture) the three species more farmed and more important commercially were considered by carrying out a total of 10 samplings. Four fish feed used in farming 7 of the 10 fish samples from aquaculture were also analyzed (Table 2): they were mainly composed by raw fish material.

Fig. 1 shows an overview of the studied area.

2.2. Chemicals and reagents

All reagents used were of pesticide grade. Acetonitrile, acetone, isooctane, *n*-hexane, petroleum ether 40–60 °C, toluene, methyl alcohol, dichloromethane, ethyl acetate, sodium sulphate and Florisil® 60–100 mesh were purchased from Carlo Erba® (Milan, Italy). The Supelclean LC-18 solid phase was from Supelco® (Bellefonte, PA, USA). Pure standards of OC pesticides were purchased from different producers, mainly in solution form, along with their certificates of analysis. Certified solutions, as single standard or mixtures, were from Dr. Ehrenstorfer® (Augsburg, Germany), AccuStandard® Inc. (New Haven, CT, USA), Supelco® (Bellefonte, PA, USA) and Riedel-de Haen/Fluka/SIGMA ALDRICH® (Switzerland). Organochlorine pesticides investigated were: aldrin, dieldrin, 4,4′-DDT, 4,4′-DDD, 4,4′-DDE, 4,4′-DDMU, 2,4′-DDT, 2,4′-DDD, 2,4′-DDE, cis-chlordane, trans-chlordane, oxychlordane, cis-nonachlor, trans-nonachlor, endrin, δ-ketoendrin, α-HCH, β-HCH, δ-HCH, γ-HCH, HCB, heptachlor, heptachlor exo epoxide, octachlorostyrene, α-endosulfan, mirex, quintozen.

The Standard Reference Material "1946 Lake Superior Fish Tissue" (fillets from adult lake trout, *Salvelinus namaycush namaycush*) was purchased from NIST®, National Institute of Standards and Technology, Gaithersburg, MD, USA. The FAPAS® Test Material T0563, Cod Liver Oil, was from The Food and Environment Research Agency, Sand Hutton, UK.

2.3. Sample preparation

Fish samples were collected directly by us or by well-trained personnel, stored on ice and immediately transported to our laboratory. Here they were subjected to freezing until the day of analysis. Three individuals or more were mostly pooled for the determination of residual organochlorine pesticides.

For sample purification and clean up the method of Di Muccio et al. (1997) was applied with some (Orban et al., 2008) and new modifications. The detailed method is explained below.

2.3.1. Extraction

After that head, tail, bone, skin and viscera were discarded (by accurately avoiding any type of cross-contamination) the edible portion was prepared as following. Fillets were homogenized in a Waring blender 38BL40 (Waring®, New Hartford, CT – USA).

Owing to their lipophilic nature the pollutants were extracted from the sample together with the fat. The extraction was carried out on approximately 15 g of homogenized fillet, exactly weighed, in a centrifuge tube by using 60 mL of acetone : petroleum ether 1 : 1 (30 mL + 30 mL). After that solvents were added the sample was homogenized for 90 s at 11 000 rpm with an Ultra Turrax homogenizer (Model T25B, IKA®, Staufen, Germany) as dispersing tool. 60 mL of a sodium chloride solution (50 g L^{-1}) were added then the tube was manually shaken for 2 min and a centrifugation step (230 *g* at 10 °C for 10 min) was conducted. Supernatant was recovered and 30 mL of petroleum ether were added in the tube followed by manually shaking and a second centrifugation. This task was repeated once more for a total of three centrifugation steps. Each time the supernatant was placed in a glass column (2 cm in diameter × 25 cm in length) filled with 25 g of sodium sulphate: in this way the supernatant was eluted through Na_2SO_4 and collected into an Erlenmeyer flask previously weighed (ether extract).

2.3.2. First purification step

To the ether extract in the Erlenmeyer flask internal standards (IS) were added. 100 μL of a solution containing 2,2′-DDE and heptachlor endo epoxide both at 304 μg L^{-1} were used. The ether extract was brought to a small volume at 40 °C by means of the Rotary Evaporator V513/R-200/B-490 (Büchi®, Switzerland) then to complete dryness (to constant weight) by manually rotating. The total amount of fat extracted was derived from the difference in weight between the empty flask (previously weighed) and the flask with the sample. The fat was carefully dissolved with 1 mL of *n*-hexane and the resulting solution was passed in a diatomaceous earth system, as follows. The hexanic solution of the fat was loaded in an Extrelut® NT3 cartridge (Merck® KgaA, Darmstadt, Germany). After 5 min the *n*-hexane was removed by passing a stream of nitrogen through the cartridge at 240 mL min^{-1} for 25 min from bottom to top by means of a rotameter (Supelco®, Bellefonte, PA, USA). Subsequently an Extrelut® NT1 cartridge has been emptied until a layer of 1 cm of diatomaceous earth remained, then 0.36 g of a C_{18} phase (Supelclean® LC-18, Supelco) was placed in the cartridge, and finally a 1.5 cm layer of diatomaceous earth was placed over the C_{18} phase.