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Distribution and trend of organochlorine pesticides in galicia coast using mussels as bioindicator organisms. Possible relationship to biological parameters

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HIGHLIGHTS

- +34 We investigated OCPs distribution in wild mussel coming from the Galician Rías.
- The level of OCPs was in the order ΣDDs (sum of DDTs and metabolites) >γ-HCH > HCB.
- Other pesticides, such as, aldrin, endrin, isodrin were not detected.
- Samples from Ría of Ferrol showed the highest levels of OCPs.
- OCPs levels decreased along the period 1998–2012 in Galician coast.

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ABSTRACT

Contamination of organochlorine pesticides (OCPs) such as DDT and its metabolites (ΣDDs), γ-HCH (hexachlorocyclohexane), HCB (hexachlorobenzene), aldrin, endrin, isodrin and trans-nonachlor were investigated in wild mussels (*Mytilus galloprovincialis*) collected from Galician Rías (Rías of Ferrol, A Coruña, Muros, Arousa, Pontevedra and Vigo) during the period 1998–2012. Biological parameters, lipid content, shell length and condition index, were also studied. The OCPs levels in the wild mussel were in the order ΣDDs > γ-HCH > HCB. The other pesticides, aldrin, endrin, isodrin and trans-nonachlor, were not detected or were below the analytical detection limit. All concentrations found in these samples were below the allowable limits for human consumption (Regulation (EC) no. 396/2005). Univariate analysis confirmed that levels of some compounds presented significant relation with biological parameters. Multivariate analysis of the OCPs levels revealed significantly differences between studied Rías, samples from Ría of Ferrol had the highest levels of these compounds (values of ΣDDs ranged from 3.9 to 4.2 ng g^{-1} ww) and samples from Ría of Arousa, the lowest levels (values of ΣDDs from 1.3 to 2.4 ng g^{-1} ww). Temporal trends showed a decrease of OCPs levels along the studied period 1998–2012 in the Galician Rías.

1. Introduction

The presence of organochlorine pesticides (OCPs) in the marine environment is of great importance due to their bioaccumulation/biomagnification power in wildlife involving a wide range of trophic levels, their persistence and the large volume of usage in the past, this could cause serious problems for years (Kennish, 1997). Various studies have proved that organochlorine compounds exhibit several types of toxicity in humans and marine organisms (Tanabe et al., 1994; Reed et al., 2007), especially the neurotoxicity. Other undesirable effects are linked to teratogenic and reproductive dysfunctions as well as endocrine disruption (Rattner, 2009). OCPs have characteristic of volatility and long-range environment transport, being widely distributed in marine environment as a consequence of uncontrolled spillage of agricultural sources, surface runoff and atmospheric deposition (Turner et al., 1986).

Biomonitoring programs are fundamental in order to know the behaviour and fate of OCPs, and to determine their spatial and temporal distribution in the marine environment. In other occasions, the regional programs allow assessing anthropogenic impact on the littoral waters and knowing baseline conditions (Chase et al., 2001; Liu and Kueh, 2005; Kljaković-Gaspić et al., 2010). Mussels have been successfully used as important indicator "sentinel" of the pollution of the marine environment in the coastal monitoring programs. Their high filtration power makes them to accumulate chemicals from surrounding water and to identify variation in

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contaminants in a specific area. They have several advantages such as the wide geographical distribution, sessile behaviour, easy sampling and resistance to stress and to a wide range of chemicals (Phillips and Rainbow, 1993; Widdows et al., 1995; Baumard et al., 1998; Chase et al., 2001). The bioaccumulation of organochlorine compounds depends on biological parameters of mussel such as, lipid content and condition index. These factors are related to physiological and reproductive conditions that vary according to the geographic sites and seasons of the year (Hummel et al., 1990).

DDT and other OCPs were widely used as a pesticide since the mid-fifties of the last century to the mid-seventies. Though, the use of many persistent OCPs were forbidden in developed countries later, the use and production of DDT were allowed to control disease vectors following the recommendations and guidelines of the WHO, when other alternatives were not available (WHO, 2007). Dicofol (<0.1% of DDT) is an insecticide that is chemically related to DDT, in 2008, it was used in three of the four Galician provinces. In Spain, dicofol will be used until 2014. In relation to γ-HCH (lindane) is a broad spectrum insecticide and fumigant that has been used on a wide range of plant eating insects. In Galicia, there is an old industrial area called Polígono de Torneiros (Porriño, Pontevedra, Spain), where a chemical factory developed its industrial activity involved in the lindane production between 1947 and 1964. At present, its soil has been declared as contaminated by γ-HCH. Most of OCPs have also been banned (Orden de 4 de Febrero 1994; Decision, 2000/801/EC). In 2008, in Spain agricultural pesticide consumption was 94549 tons, 3% of which corresponds to Galicia. The most widely used pesticides in Galicia were herbicides (38%) followed by fungicides (33.3%) and insecticides (23.1%) (Asociación empresarial, 2008).

The Galician littoral is a marine environment where the 95% of Spanish mussel occurs. In Galicia (NW, Spain), there are a series of estuarine bays (called Rías) that are old river valleys taken over the sea. The water of these estuarine bays has an excellent quality for the growing of bivalve mollusc due to high concentration of nutrients and moderate temperatures. Galicia is the largest mussel producers in the world, this causes that Galician administrative authorities have a duty to control and ensure the quality of shellfish that is produced in its coast.

Only few studies have investigated the presence of OCPs in Galician marine environment where they have occurred in relatively low levels (Alvarez Piñeiro et al., 1995; Vidal-Liñán et al., 2010; Carro et al., 2012; Suarez et al., 2013).

The present study aims to asses the occurrence and distribution of the main organochlorine pesticides compounds, such as γ-HCH (Hexachlorocyclohexane), HCB (Hexachlorobenzene), DDTs (op′ and pp′-dichlorodiphenyltrichloroethane) and their metabolites, pp′-DDE and pp′-DDD, in *Mytilus galloprovincialis* collected in the Galicia coast. This research is part of a monitoring program, fifteen years, focused on POPs (persistent organic pollutants) in wild mussels from six Rías in Galicia (N.W., Spain), Ría of Ferrol, Ría of A Coruña, Ría of Muros, Ría of Arousa, Ría of Vigo and Ría of Pontevedra. The main objective of this monitoring was to control the water quality for shellfish production (Regulation (EC) No. 113/2006). The influence of mussel physiological conditions on the OCPs bioaccumulation was also investigated.

2. Materials and methods

2.1. Reagents and standards

Dichloromethane, diethyl ether, *n*-pentane and isooctane for organic trace analysis were supplied by Merck (Darmstadt, Germany). Aluminium oxide, silica gel and anhydrous sodium sulphate were also supplied by Merck. Analytical reagent grade OCPs (γ-HCH, HCB, pp′-DDE, pp′-DDD, op′-DDT, pp′-DDT, aldrin, endrin, isodrin and trans-nonachlor) were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

For quantitative gas chromatographic determinations, calibration was carried out at five concentration levels for each congener spanning the range of 1–200 ng g^{-1} and using CB 155 (500 ng g^{-1}) as an internal standard.

2.2. Samples

Sample collection was carried out at 25 sampling points from several Galician Rías (N.W. Spain). Wild mussels (*M. galloprovincialis*) were collected from 3 points in the Ría of Ferrol (Fer 1, Fer 2 and Fer 3), 2 points in the Ría of A Coruña (Cor 1 and Cor 2), 3 points in the Ría of Muros (Mur 1, Mur 2 and Mur 3), 9 points in the Ría of Arousa (Aro 1 to Aro 9), 3 points in the Ría of Pontevedra (Pon 1, Pon 2 and Pon 3) and 5 points in the Ría of Vigo (Vig 1 to Vig 5) in the period from 1998 to 2012 (see Fig. 1). All samples were collected at the same time in the year, during the month prior to spawning of mussel.

2.3. Procedure

Thirty individual mussels of similar sizes (50–75 mm) were used for each analysis. Homogenates of the mussel flesh were frozen (−30 °C) and freeze-dried. 5 g of homogenized mussel sample was extracted in a Soxhlet apparatus with a mixture of dichloromethane–pentane (1–1) for 8 h. An aliquot of the extract was used to determine gravimetrically the lipid content. Interfering lipids were removed from a suitable portion of the extract by chromatography over alumina (6% deactivated) and target compounds were eluted with *n*-pentane. OC pesticides fraction was separated by chromatography on silica (1% deactivated) eluting with a mixture of isooctane–diethyl ether (8.5–1.5 v/v). Extract was concentrated under nitrogen stream to near dryness and then redissolved in 1 mL of isooctane. PCB 155 was added as an internal standard prior to analysis by gas chromatography.

Condition index (CI) was calculated as the ratio between shell weight and body weight of each sample (thirty individual mussels).

2.4. Apparatus

The purified extracts were analysed by a gas chromatography using a Perkin–Elmer Autosystem Gas Chromatograph equipped with ^{63}Ni electron capture detector (GC–ECD). A TRB-5 (Teknokroma, Spain) 5% diphenyldimethyl siloxane capillary column (60 m × 0.20 mm i.d. × 0.4 μm phase thickness) was used. The chromatographic conditions were: the column temperature program was 90 °C (3 min)–215 °C (40 min) at a rate of 30 °C min^{-1} and 275 °C (30 min) at a rate of 5 °C min^{-1}. The injector temperature (splitless mode, 1.8 min.) was set at 270 °C. The ECD temperature was set at 365 °C. The carrier gas was hydrogen and the make-up gas was argon/methane, both purchased from Air Liquid (Spain).

The identification of OCPs was carried out on a Varian Saturn 2000 Gas Chromatograph-Ion Trap Detector Mass Spectrometer (ITD). The experimental conditions and capillary column were identical to those described before. The carrier gas was helium (Air Liquid, Spain). A non-resonant wave form was selected. The mass spectrometer conditions were, the emission current of filament, 80 μA, the electron multiplier voltage offset, +200 V, the maximum ionisation time, 25000 μs. The CID (collision-induced dissociation) amplitude was determined using the automated method development (AMD, Varian software). The parent ion mass was selected in function of maximum intensity. The excitation