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Organochlorine pesticide residues in muscle tissue of rainbow trout, *Oncorhynchus mykiss*, taken from four fish farms in León, Spain

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Levels of nine organochlorine pesticides (lindane, heptachlor epoxide, aldrin, dieldrin, endrin, o,p′*-TDE,* p,p′*-TDE,* p,p′*-DDE and* p,p′*-DDT) were determined in muscle samples of rainbow trout,* Onchorhynchus mykiss, *collected from four fish farms in the province of León, in the north-west of Spain (Europe). The highest incidence percentage was for lindane (67.5%) and heptachlor epoxide (55.0%). Organochlorine residue levels detected contributed slightly to acceptable daily intakes (ADIs) established by the Food and Agriculture Organization of the United Nations and the World Health Organization (lindane 0.22–2.3%; ΣDDT 0.05–0.46%; heptachlor epoxide 10.0–71.4% and Σdieldrin 16.7–33.3% assuming 300 g of trout muscle as a mean daily intake). The highest concentration found was for heptachlor epoxide (0.043 μg/g).*

Keywords: organochlorine pesticide, residues, rainbow trout

Introduction

Organochlorine pesticides are very persistent compounds and their persistence in the environment is measured in years. As a result of their persistence and high capacity for accumulation in living organisms, the use of the majority of organochlorine pesticides has been banned or restricted in the developed countries. However, these compounds are still present in the environment and/or in animal tissues.

European Union (EU) Legislation (1992) allows the use of lindane and, severely restricted, endrin and aldrin but, as in Spain, each country can have additional rules. In Spain, at the present moment, only lindane can be used without restrictions. Nevertheless, legislation (Ministerio de Sanidad y Consumo 1994) allows the occasional use of any of these compounds if resistance to other insecticides appears. For the reasons mentioned above, these compounds are currently detected, mainly in biological samples (Fernandez *et al.* 1993, Hernandez *et al.* 1993, Losada *et al.* 1996).

The purpose of this paper was to report the concentrations of nine organochlorine pesticides in muscle samples of rainbow trout (*Onchorhynchus mykiss*) taken from four fish farms in León, in the north-west of Spain. The data obtained will provide some information about their implications for human health. Due to the large annual production of these fish farms (ranging from 30 to 78 tons), it is important to know how the detected residue levels contribute to the ADI established by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO 1985) according to European Union Legislation (1991).

In order to establish if any change had occurred since 1987 in organochlorine contamination in trout, we have also compared data obtained in this study with those reported by Teran and Sierra (1987) in trout, *Salmo trutta fario*, caught in the same or contiguous rivers to this study.

Material and methods

Forty muscle samples of rainbow trout (approximately 50% males and 50% females) were obtained

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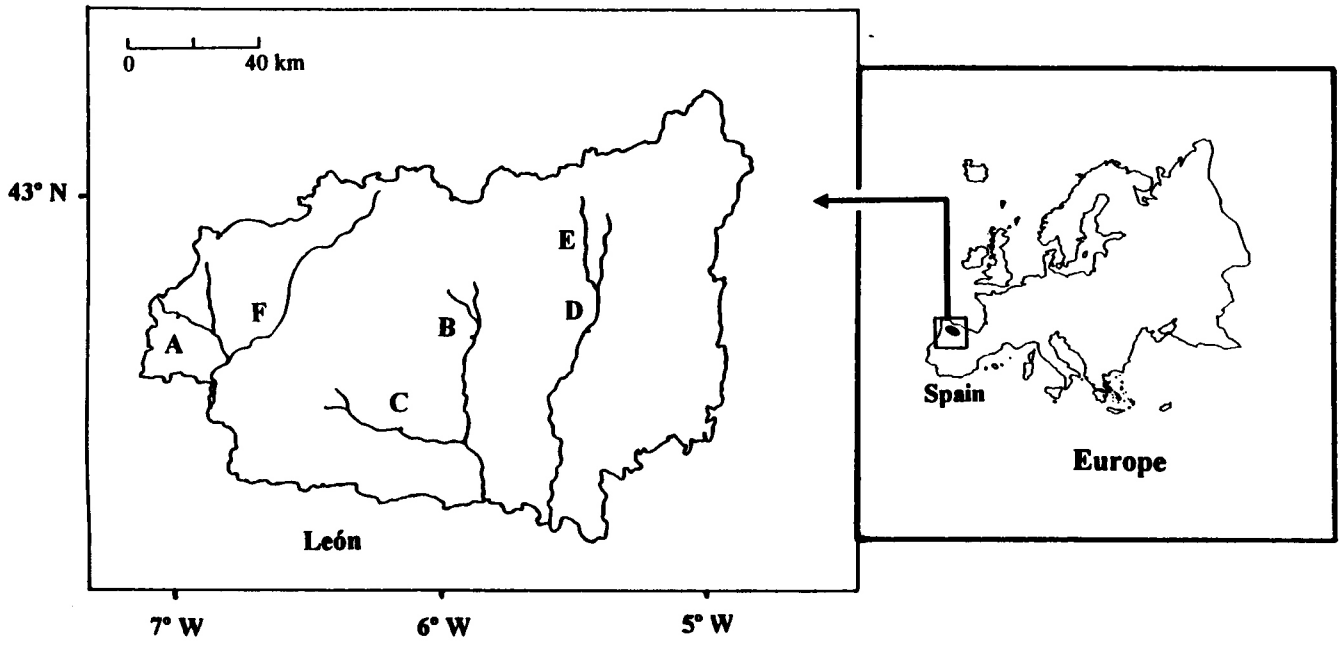

Figure 1. Map of León showing sampling locations: A—Trabadelo (Valcarce river); B—Carrizo de la Ribera (Orbigo river); C—Castrillo de la Valduerna (Duerna river); D—Castrillo de Porma (Porma river); E—Curueño river, and F—Sil river.

during the winter of 1993 from four fish farms in the province of León (Trabadelo [A], Carrizo de la Ribera [B], Castrillo de la Valduerna [C] and Castrillo de Porma [D]) (figure 1). Trout weights ranged from 217.6 to 257.4 g (fish farm A); 200.6 to 295.7 g (fish farm B); 210.4 to 310.6 g (fish farm C) and 205.4 to 311.5 g (fish farm D). A sample of homogenized muscle (30 g) was removed from each trout. Samples were obtained from entire fillets which were stored at −20°C until they were analysed. The lipid percent of these samples ranged from 1.51 to 2.53%. Fish farms to be sampled were selected due to their large production (their annual production is 31 tons in fish farm A, 30 tons in fish farm B, 78 tons in fish farm C and 40 tons in fish farm D) and with the aim of comparing the residue levels detected with those obtained in a previous study carried out in trout (*Salmo trutta fario*) taken from the same or contiguous rivers in 1987.

Organochlorine residues were extracted and cleaned-up according to the method provided in 1990 by the Association of Official Analytical Chemists (Sawyer *et al.* 1990). The cleaned-up extracts were completely evaporated under a gentle stream of nitrogen and 1 ml of hexane was added for reconstitution. All reagents used were of pesticide analysis grade and their purity was carefully checked as indicated in the aforesaid method.

The samples were tested for the residues of nine organochlorine pesticides: lindane, heptachlor epoxide, aldrin, dieldrin, endrin, *o,p'*-TDE, *p,p'*-TDE, *p,p'*-DDE and *p,p'*-DDT. Identification and quantification of residues were made by gas chromatography against a reference standard containing these pesticides. The reference standard pesticides were purchased from Dr Ehrenstorfer GmbH, Scharlau (Augsburg, Germany).

A Hewlett Packard gas chromatograph equipped with a ^{63}Ni electron capture detector system and a computer was used. The operating conditions were as follows: column a, 1.83 m long and 6.4 mm internal diameter containing a 10% coating of DC-200 on 80–100 mesh Chromosorb WHP (Sawyer *et al.* 1990). A second column (b) packed with 1.95% QF-1/1.5% OV-17 on 100/120 Chromosorb W (AW/OMCS) was used to confirm the identity of the organochlorine residues when necessary. The operating temperatures were: inlet, 240°C (column a) and 220°C (column b); detector, 300°C; column a, 220°C (21 min) and 230°C (10 min); column b, 180°C (15 min) and 190°C (20 min), with an intermediate temperature increase,

in both cases, of 10°C/min. The carrier gas was 5% argon–methane at a flow rate of 24 ml/min and the sample volume injected was 3 µl.

The peaks were identified by comparison of retention times with those of the standard and tolerance allowed was ±5% of retention times. The detection limit was established as 0.0005 µg/ml for dieldrin and *o,p'*-TDE; 0.001 µg/ml for lindane, heptachlor epoxide, aldrin, endrin and *p,p'*-TDE and 0.005 µg/ml for *p,p'*-DDE and *p,p'*-DDT. Residue concentrations in samples were calculated by comparing the peak areas in the sample with those obtained with the mixed standard solution.

Recoveries of organochlorine compounds from fortified samples were: 80.4% (*p,p'*-DDE), 80.6% (endrin), 83.9% (dieldrin), 88.7% (aldrin), 87.4% (*p,p'*-TDE), 93.5% (*p,p'*-DDT) and 100.0% (lindane, heptachlor epoxide and *o,p'*-TDE). Residue levels of pesticides were corrected according to their recoveries.

The influence of the fish farm on residual levels detected for each pesticide was analysed for statistical significance by using the one-way analysis of variance (ANOVA), when possible. When the results were significant, Duncan's test was used to evaluate differences between data sets and $P \leq 0.05$ was taken as the level of significance for all analyses.

Results

The mean concentrations (in µg/g wet weight), ranges and incidence percentages of organochlorine residues obtained in muscle samples from the four fish farms are shown in table 1. ΣDDT refers to the sum of *o,p'*-TDE, *p,p'*-TDE, *p,p'*-DDE and *p,p'*-DDT residues. Taking into account all the samples, some of the nine pesticides were detected in each one of them. Lindane was the most frequently detected pesticide (67.5%), followed by heptachlor epoxide (55.0%) while endrin, dieldrin and *o,p'*-TDE were never detected.

The highest mean residue concentrations were for heptachlor epoxide in fish farms A (0.087 µg/g), B (0.016 µg/g) and D (0.074 µg/g), and for *p,p'*-DDT in fish farm C (0.032 µg/g). In fish farm A the lindane concentration was also high (0.053 µg/g). Considering all data, the highest mean concentration was also for heptachlor epoxide (0.043 µg/g).

The results of the one-way ANOVA analysis carried out to determine the influence of geographical area on the residual level obtained for each pesticide and on the total load, showed significant differences only for heptachlor epoxide between Trabadelo (A) and Carrizo de la Ribera (B) and between Trabadelo (A) and Castrillo de la Valduerna (C).

EU legislation for organochlorine residue levels in trout has been established according to the ADI proposed by FAO/WHO (1985) (expressed in µg/kg body weight): 10 for lindane, 0.5 for heptachlor epoxide; 0.1 for Σdieldrin (aldrin + dieldrin) and 20 for ΣDDT. In order to calculate the amount of pesticide ingested we have used the mean data obtained from each fish farm, and have assumed 300 g of trout muscle as a mean daily intake for a man with a body weight of 70 kg. So, the organochlorine pesticide amount ingested is very far from the ADI for lindane (from 44 to 470 times lower, approximately) and ΣDDT (from 220 to 2100 times lower, approximately). Heptachlor epoxide levels are 1.4–10 times lower than the value of the ADI and Σdieldrin concentrations were three to six times lower. It must be noted that percent occurrence of ΣDDT and Σdieldrin (10–40%) was low in these samples; for heptachlor epoxide it was 20–80% and 30–90% for lindane.

Discussion

In a previous study (Teran and Sierra 1987) carried out in muscle samples of trout (*Salmo trutta fario*) taken from the same or nearby rivers (Orbigo [B], Curueño [E] and Sil [F] rivers) (see figure 1), incidence percentages were higher than those reported in this study. The most frequently occurring pesticides detected by Teran and Sierra (1987) were heptachlor epoxide (95.45%) and ΣDDT (72.73%).

Miller *et al.* (1992) investigated organochlorine compound levels (dieldrin, DDT and analogues, *cis* and *trans* chlordane, and *cis* and *trans* nonachlor) in muscle tissue samples of trout from Lake Michigan and Lake Superior (USA). The highest incidence percentage was for dieldrin (96%) in Lake Michigan trout and for *p,p'*-DDT (100%) and dieldrin (94%) in Lake Superior samples.

Teran and Sierra (1987) found mean concentrations of heptachlor epoxide (0.007 µg/g) six times lower

Table 1. Mean, range (in μg/g wet weight) and incidence (%) of organochlorine pesticides.

	Fish farm A	Fish farm B	Fish farm C	Fish farm D	Mean load
Lindane					
	0.053	0.005	0.005	0.014	0.024
	(0.002–0.441)	(0.002–0.014)	(0.002–0.009)	(0.001–0.060)	(0.001–0.441)
	90%	80%	30%	70%	67.5%
Heptachlor epoxide					
	0.087	0.016	0.012	0.074	0.043
	(0.023–0.150)	(0.003–0.040)	(0.004–0.024)	(0.008–0.173)	(0.003–0.173)
	20%	70%	50%	80%	55%
Aldrin					
	0.007				
	(0.003–0.015)	—	(0.004–0.011)	—	(0.003–0.015)
	40%	10%	40%	10%	25%
Dieldrin					
	—	—	—	—	—
Endrin					
	—	—	—	—	—
p,p'-DDE					
		0.015	0.011		0.013
	—	(0.011–0.019)	(0.010–0.012)	—	(0.010–0.019)
		20%	20%		10%
o,p'-TDE					
	—	—	—	—	—
p,p'-TDE					
	0.002	0.003		0.009	0.006
	—	—	—	(0.009–0.009)	(0.002–0.009)
	10%	10%		20%	10%
p,p'-DDT					
			0.032		0.032
	—	—	(0.018–0.047)	—	(0.018–0.047)
			20%		5%
ΣDDT					
	0.002	0.011	0.022	0.009	0.014
	—	(0.003–0.019)	(0.010–0.047)	(0.009–0.009)	(0.002–0.047)
	10%	30%	40%	20%	25%
Mean load	0.068	0.019	0.019	0.072	0.045

than those recorded in this study. The levels of lindane detected (0.008 μg/g) were also three times lower than those found in this study. In contrast, aldrin (0.018 μg/g) and ΣDDT (0.673 μg/g) were detected at higher concentrations by Teran and Sierra (1987): three and 50 times higher, respectively. The important decrease in endrin, dieldrin, *o,p'*-TDE and ΣDDT levels can be attributed to the severe restrictions on the use of these organochlorine pesticides.

Heptachlor epoxide concentrations detected in this study were also higher than those reported by other authors in freshwater species of fish. In muscular fat samples from rainbow trout and brown trout caught in a river of Turin (Italy), Forneris *et al.* (1986) found heptachlor epoxide concentrations of 1.5 ng/g and 0.8 ng/g, respectively which are lower than those shown in this study.

Miller *et al.* (1992) found higher concentrations than those reported in this study for *p,p'*-TDE, *p,p'*-DDE, *p,p'*-DDT and dieldrin in trout muscle samples from Lake Michigan and Lake Superior. In this study the

concentrations of these pesticides ranged from 0.05 to 0.24 μg/g (p,p'-TDE); 0.34 to 6.49 μg/g (p,p'-DDE); 0.05 to 0.33 μg/g (p,p'-DDT) and 0.02 to 0.41 μg/g (dieldrin).

In 1993, Miller *et al.* also reported higher levels in rainbow trout muscle samples from Lake Michigan for ΣDDT (0.38 μg/g) than our data (0.014 μg/g) and for dieldrin (0.07 μg/g), which was not detected in our study.

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