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Organochlorine Pesticide Residues in Rainbow Trout, *Oncorhynchus mykiss,* Taken from Four Fish Farms in León, Spain

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Chlorinated pesticides possess high chemical stability and strong lipophilic properties. Their use was restricted in Spain more than eighteen years ago, but they still exist as pollutants due to their occasional use and their persistence in the environment. Aquatic organisms, especially fish, can accumulate organochlorine residues directly from water through their respiratory processes and also from food. The residual levels detected in their tissues may reflect the environmental contamination. In this way, liver and kidney samples were used because they are the elimination organs for pesticides,and brain samples were selected because in this organ is where organochlorine toxicity is manifested.

The main purpose of the present study was to establish the organochlorine pesticide contamination in rainbow trout *(Oncorhynchus mykiss)* taken from fish farms in Leon, in the North-West of Spain. Data obtained will provide some information about environmental contamination. Another objective was to investigate whether modifications in levels of these contaminants have occurred since 1987 by comparison with data obtained in a previous study carried out in trout, *Salmo trutta fario* (Terán and Sierra 1987).

MATERIALS AND METHODS

During the winter of 1993, 40 rainbow trout, 10 from each fish farm, *(Oncorhynchus mykiss)* were collected from four different fish farms in the province of León (Trabadelo [A], Carrizo de la Ribera [B], Castrillo de la Valduema [C] and Castrillo de Porma [D]) (Fig. 1). The trout collected in each fish farm were approximately 50% males and 50% females and their 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). The sampling locations were selected to assess an organochlorine pesticide contamination study within a wide geographical area and to compare 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.

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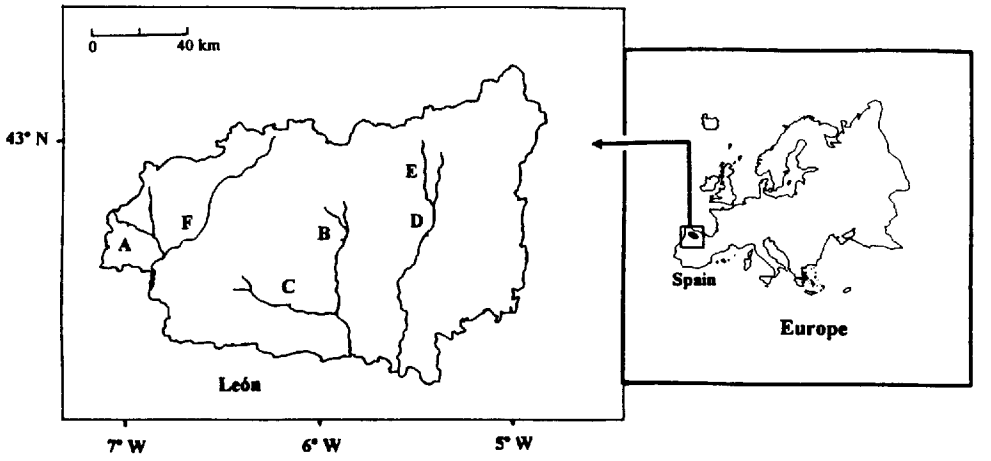

Figure 1. Map of León showing sampling, locations: A.- Trabadelo (Valcarce river); B.- Carrizo de la Ribera (Orbigo river); C.- Castrillo de la Valduerna (Duerna river); D.- Castrillo de Porma (Porma river); E.- Curueño river and F.- Sil river.

A total of 120 samples of liver, kidney and brain were obtained and all of them were stored at -20°C until they were analyzed. The lipid percent of these samples ranged from 2.6 to 3.2% in liver; from 4.6 to 4.8% in kidney and from 5.9 to 6.9% in brain. Extraction of organochlorine pesticides and clean-up of hexane extracts were carried out according to the method provided 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. The residues were analyzed by electron capture gas chromatography by using a Hewlett Packard gas-chromatograph fitted with a Ni^{63} electron capture detector and a computer.

The operating conditions were as follows: column a) 6 feet long and 1/4 inch 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/l.5% OV-17 on 100/120 mesh 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 minutes) and 230°C (10 minutes); column b) 180°C (15 minutes) and 190°C (20 minutes), 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 sample volume injected was 3 µL.

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. A reference standard containing these pesticides was used to identify and quantify the levels of residues. The peaks were identifed by comparison of retention times with those of the standards, and tolerance allowed was ± 5% of retention times. The detection limit was established in 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 comparison of the sample peak areas with those in corresponding standards.

Recoveries of organochlorine compounds from fortified samples were: 80.3% (dieldrin), 86.1% (lindane), 88.6% (aldrin and p,p'-DDT), 91.0% (p,p'-TDE and p,p'-DDE), 92.5% (heptachlor epoxide) and 100% (o,p'-TDE and endrin) in brain; 81.3% (p.p'-DDE), 82.4% (dieldrin and endrin). 84.0% (p,p'-TDE), 86.2% (p,p'-DDT), 95.6% (lindane) and 100% (heptachlor epoxide, aldrin and o,p'-TDE) in kidney; 83.6% (p,p'-DDE), 86% (p.p'-TDE), 87.5% (p.p'-DDT and dieldrin) and 100% (lindane, heptachlor epoxide, aldrin, o,p'-TDE and endrin) in liver.

Fish farm influence in residual levels detected for each pesticide in the different samples (liver, brain and kidney) were analyzed for statistical significance by using the one-way analysis of variance (ANOVA), when possible. In the same way, the loads obtained for each pesticide were compared to detect possible fish farm influences. When the results were significant, Duncan test was used to evaluate differences between data sets and a $P \leq 0.05$ was taken as the level of significance for all analyses.

RESULTS AND DISCUSSION

The mean concentrations, ranges and incidence percentages of organochlorine residues in the three types of samples expressed in ppm by weight of wet sample are shown in Tables 1 to 4. In these tables the results for each fish farm are included. ΣDDT refers to the sum of o,p'-TDE; p,p'-TDE, p,p'-DDE and p,p'-DDT residues. Table 5 shows the same parameters, but calculated from the four fish farms data, grouping all samples according to the type of tissue.

Only 3 of the 120 samples analyzed showed no pesticide residues. Endrin was not detected in any sample from three fish farms (A, B and D) and it was only present in a single kidney sample from fish farm C. Dieldrin was not detected in any sample from fish farms A and C either. Taking into account all samples, the highest incidence percentage was for heptachlor epoxide (80% in brain 95% in kidney and 90% in liver). Albright et al. (1980) indicated that elimination of heptachlor epoxide was very slow in cutthroat trout *(Salmo clarki)* and it would explain the high incidence

Table 1. Mean, range (ppm) and incidence (%) of organochlorine pesticides in trout from Trabadelo (Valcarce river) (A).

	Brain	Liver	Kidney	Mean Load
Lindane	0.237 (0.071-0.329) 50%	0.021 (0.008-0.032) 80%	0.066 (0.028-0.116) 80%	0.088
Heptachlor epoxide	0.194 (0.100-0.347) 80%	0.047 (0.013-0.153) 90%	0.218 (0.062-0.495) 100%	0.152
Aldrin	0.111 (0.064-0.158) 20%	—	0.053 (0.046-0.059) 20%	0.073
Dieldrin	—	—	—	—
Endrin	—	—	—	—
ΣDDT	0.649 (0.013-2.287) 70%	0.029 (0.001-0.147) 60%	0.059 (0.003-0.154) 90%	0.237
Mean Load	0.750	0.076	0.334	0.387

Table 2. Mean, range (ppm) and incidence (%) of organochlorine pesticides in trout from Carrizo de la Ribera (Órbigo river) (B).

	Brain	Liver	Kidney	Mean Load
Lindane	0.161 (O.124-0.194) 70%	0.021 (0.006-0.036) 90%	0.026 (0.017-0.035) 50%	0.067
Heptachlor epoxide	0.860 (0.088-2.718) 70%	0.114 (0.045-0.176) 100%	0.339 (0.026-1.558) 100%	0.370
Aldrin	0.096 (0.087-0.106) 20%	—	0.081 (0.069-0.093) 20%	0.089
Dieldrin	0.094 — 10%	—	—	0.094
Endrin	—	—	—	—
ΣDDT	1.533 (0.133-4.798) 70%	0.081 (0.001-0.334) 50%	0.025 (0.003-0.053) 70%	0.458
Mean Load	2.019	0.174	0.385	0.800

Table 3. Mean, range (ppm) and incidence (%) of organochlorine pesticides in trout from Castrillo de la Valduerna (Duerna river) (C).

	Brain	Liver	Kidney	Mean Load
Lindane	0.065 (0.045-0.082) 50%	0.014 (0.008-0.038) 100%	0.047 (0.028-0.070) 60%	0.034
Heptachlor epoxide	0.320 (0.079-0.981) 80%	0.064 (0.009-0.151) 100%	0.186 (0.029-0.861) 100%	0.175
Aldrin	0.068 — 10%	—	—	0.068
Dieldrin	—	—	—	—
Endrin	—	—	0.005 — 10%	0.005
ΣDDT	0.788 (0.018-1.897) 50%	0.026 (0.013-0.058) 80%	0.141 (0.012-0.439) 90%	0.194
Mean Load	0.689	0.098	0.342	0.376

Table 4. Mean, range (ppm) and incidence (%) of organochlorine pesticides in trout from Castrillo de Porma (Porma river) (D).

	Brain	Liver	Kidney	Mean Load
Lindane	0.121 (0.046-0.332) 50%	0.025 (0.015-0.053) 50%	0.034 (0.029-0.039) 20%	0.064
Heptachlor epoxide	0.464 (0.144-1.266) 90%	0.094 (0.010-0.231) 70%	0.252 (0.124-0.449) 80%	0.282
Aldrin	0.063 (0.029-0.119) 60%	—	—	0.063
Dieldrin	0.418 (0.321-0.515) 20%	—	—	0.418
Endrin	—	—	—	—
ΣDDT	0.628 (0.027-1.823) 70%	0.053 (0.028-0.114) 80%	0.057 (0.031-0.092) 60%	0.254
Mean Load	1.155	0.121	0.270	0.483

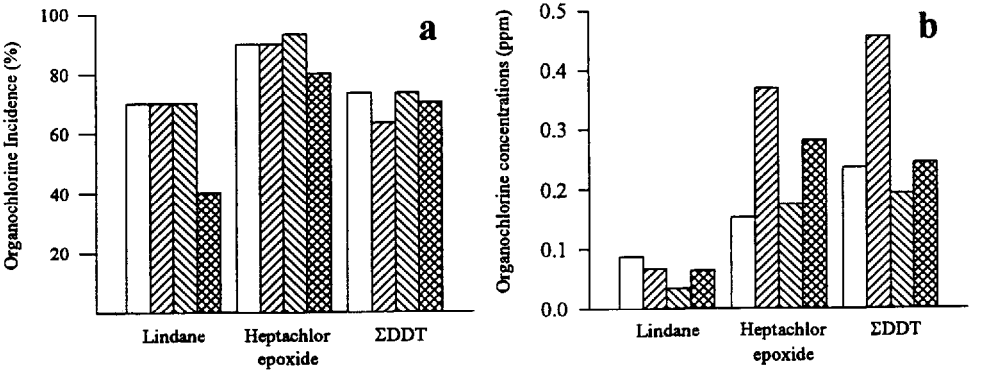

Figure 2. Percent detection and average concentrations of lindane, heptachlor epoxide and ΣDDT in brain liver and kidney tissue (combined levels) in four fish farms: A: ☐ B: ▨ C: ▩ and D: ■

detected for this compound. ΣDDT was the most frequently found pesticide in brain (65%) and kidney (77.5%) samples after heptachlor epoxide, and lindane in liver samples (80%). As shown in Figure 2 (a), incidence values were very similar for the most frequently detected pesticides (lindane, heptachlor epoxide and ΣDDT) in the four fish farms.

We have found no studies on organochlorine pesticide incidence in rainbow trout from fish farms in Spain. Terán and Sierra (1987) in a study carried out in trout *(Salmo trutta fario)* taken from the same or next rivers (Órbigo [B], Curueño [E] and Sil [F] rivers) to those chosen for this study (see Fig. 1), found the highest incidence percentage for ΣDDT in liver and kidney (95.45% and 81.81%, respectively) and for ΣDDT and aldrin in brain (100%).

Forneris et al. (1986) determined organochlorine pesticide residues in some species of freshwater fishes, including two trout species *(Salmo trutta* and *Oncorhynchus mykiss),* caught in rivers and streams in the province of Turin (Italy). These authors carried out the incidence study using data from all samples and they do not provide incidence data for each species. The highest incidence percentages were for aldrin (95.8%) and for DDT (79.2%).

Considering the most frequently detected pesticides (lindane, heptachlor epoxide and ΣDDT), the highest mean residue concentrations in the four fish farms were for ΣDDT: 0.649 ppm (A); 1.533 ppm (B); 0.788 ppm (C) and 0.628 ppm (D). These values were detected in brain samples in all cases and p,p'-TDE was the fraction detected at a highest concentration, except in fish farm B where the highest concentration was for p,p'-DDT. Figure 2 (b) includes the lindane, heptachlor epoxide and ΣDDT load in the four fish farms.

Table 5. Mean range and incidence percentage (ppm) of organochlorine pesticides in rainbow trout from the four fish fins.

	Brain	Liver	Kidney	Mean Load
Lindane	0.147 (0.045-0.332) 55%	0.020 (0.006-0.053) 80%	0.048 (0.017-0.116) 52.5%	0.063
Heptachlor epoxide	0.447 (0.079-2.718) 80%	0.079 (0.009-0.231) 90%	0.249 (0.026-1.558) 95%	0.245
Aldrin	0.078 (0.029-0.158) 27.5%	—	0.067 (0.046-0.093) 10%	0.073
Dieldrin	0.310 (0.094-0.515) 7.5%	—	—	0.310
Endrin	—	—	0.005 — 2.5%	0.005
ΣDDT	0.908 (0.013-4.798) 65%	0.045 (0.001-0.334) 67.5%	0.075 (0.003-0.439) 77.5%	0.286
Mean Load	1.130	0.117	0.334	0.511

In Table 5, where all data are summarized, it can be appreciated that the highest concentration is also for ΣDDT (0.908 ppm). Terán and Sierra (1987) had also found the highest concentration for ΣDDT (1.285 ppm) in brain, being p,p'-DDT the pesticide detected at a highest concentration (1. 160 ppm). Lindane and heptachlor epoxide residue levels were similar in both studies, except for heptachlor epoxide concentration in brain (0.035 ppm) which was 12.8 times higher in this study. Aldrin, dieldrin, endrin and ΣDDT levels were lower in the present study. The general decrease in organochlorine residues observed may be attributed to the restrictions on the use of pesticides.

Finally, in order to evaluate the influence of geographical area on the load obtained for each pesticide and on the total load, an analysis of variance (ANOVA) was used. No significant differences were found in Σ dieldrin, ΣDDT and total organochlorine pesticide load. Significant differences were found in heptachlor epoxide residues between Carrizo de la Ribera (B) and the other fish farms, except with Castrillo de Porma (D). Lindane levels showed significant differences between Trabadelo (A) and Castrillo de la Valduerna (C).

The results of another ANOVA analysis carried out to evaluate the effect of the geographical area on the residue levels found in each tissue are shown in Table 6.

Table 6. Results of ANOVA analysis carried out to evaluate the effect of the geographical area on the residue levels found in each tissue.

	Brain	Liver	Kidney
Lindane	A and C; A and D	C and D	A and B
Heptachlor epoxide	A and B	A and B	N.S.
Aldrin	N.S.	N.P.	N.S.
Dieldrin	N.P.	N.P.	N.P.
p,p'-DDE	N.S.	A and B; B and D	A and C; B and D C and D
o,p'-TDE	N.S.	N.S.	N.S.
p,p-TDE	N.S.	N.S.	N.S.
p,p'-DDT	A and B; B and C B and D	N.S.	N.S.
ΣDDT	N.S.	N.S.	B and C
Total	A and B	A and B	N.S.

N.S.: No significative differences ($P \leq 0.05$); N. P.: No statistical analysis performed.
A: Trabadelo fish farm; B: Carrizo de la Ribera fish farm; C: Castrillo de la Valduerna fish farm; D: Castrillo de Porma fish farm.

The results presented above suggest that there has been a decline in organochlorine residues in trout since 1987. It can be also observed that residues are mainly accumulated in brain and at a lower rate in the kidney. This fact was also observed in the previous study carried out by Terán and Sierra (1987).

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