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Lindane toxicity on early life stages of gilthead seabream (*Sparus aurata*) with a note on its histopathological manifestations

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Abstract

Eggs/embryos and larvae were exposed to nominal concentrations ranging from 0.1 to 10 mg/L lindane. High percentage of mortality was observed in larvae exposed to 1 mg/L (76.38%) and in embryos exposed to 10 mg/L (81.98%) of lindane at 24 h exposure. The acute toxicity expressed as LC_{50} 48-h was 0.122 mg/L for embryos and 0.318 mg/L for larvae. Larvae alterations included weak swimming, incapacity to respond to external stimuli, uncoordinated movements, trembling, myoskeletal defects, opaque skin and exophthalmia. Mucous epithelium of the digestive tissue showed a severe alteration with hypertrophy and desquamation of mucous cells. A high cellular disorganization in the renal and hepatic tissue is observed. Results obtained showed the sensitivity of *Sparus aurata* early life stages to lindane and the presence of sublethal effects like histopathological alterations; therefore, the relevance of pesticides substances control in the aquatic environment.

Keywords: Toxicity; Lindane; Early life stages; *Sparus aurata*; LC_{50}; Histopathology

1. Introduction

Organochlorine insecticides were introduced in the decade following World War II, which were used extensively in Europe, U.S.A., and other developed countries in 1970s. Nowadays, there exists a wide bibliography about the accumulation and persistence in soils and aquatic sediments of this chemical, their potential to be taken up by animal tissues and to be accumulated in birds, mammals and even in humans (Wiktelius and Edwards, 1997).

Lindane (hexachlorocyclohexane γ-isomer) is an organochlorine insecticide that has been used on a wide range of soil-dwelling and plant-eating (phytophagous) insects. It is commonly used on a wide variety of crops, in warehouses, and in public health to control some diseases brought about by insects. Other applications are in the manufacture of lotions and shampoos for the control of lice and mites in humans. Also, it may be found in formulations of fungicides. It is available as a suspension, emulsifiable concentrate, powder, and ultra low volume (ULV) liquid (EXTOXNET, 1996).

Lindane is very stable in both fresh- and salt-water environments, and it is resistant to photodegradation (Kidd and James, 1991). It disappears from the water by secondary mechanisms such as adsorption and absorption by sediment, flora and fauna, in the case of fishes through gills, skin, and food (Ulman, 1972).

Lindane is highly toxic to aquatic organisms (EXTOXNET, 1996). In fish reported 96-h LC_{50} values range from 0.0017 to 0.09 mg/L in trout (rainbow, brown and lake), coho salmon, carp, fathead minnow, bluegill, largemouth bass, and yellow perch (Johnson and Finley, 1980). In invertebrates, 96-h LC_{50} values were: in Daphnia, 0.46 mg/L and in *Pteronarcys* sp. (stone flies), 0.0045 mg/L (Johnson and Finley, 1980). The bioconcentration factor for the compound is 1400 times water concentrations indicating significant bioaccumulation (Ulman, 1972).

The toxicity is a complex process depending on many factors such as species, age, body weight, stage in the life cycle, feeding conditions, diet composition (Boyd and Campbell, 1983; Hichie and Dixon, 1987; Marking et al., 1984), metabolism rate of the

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organism, temperature, salinity, dissolved oxygen of the water, etc. (Braunbeck and Segner, 1992). The results of life cycle toxicity tests on fish indicate that embryos and larvae are the most sensitive stages for different contaminants (Ensenbach and Nagel, 1995).

The toxicity of lindane on juvenile and adult fish, its accumulation within tissues and its impacts on physiological mechanisms have been well studied (Geyer et al., 1994; Khalaf-Allah, 1999; Salvado et al., 2006), but the information relative to the effects of lindane on aquatic organisms exposed during the earliest life stages is limited.

The teleost *Sparus aurata* is one of the most abundant and representative species of the Atlantic and Mediterranean coasts (Arias and Drake, 1990). Currently, the culture of the teleost *S. aurata* has reached great economic and commercial interest (FAO, 1997). Due to its wide distribution, commercial importance and the wide knowledge about this species, in the past years *S. Aurata* is being using to test the toxicity of chemicals used in aquaculture or pollutants that reach the marine environment (Del Valls et al., 1998; Hampel and Blasco, 2002; Dimitriou et al., 2003; Arufe et al., 2004a,b). In this study, we assess the acute toxicity of lindane on early life stages of gilthead seabream and the associated alterations with a note on its histopathological manifestations.

2. Materials and methods

2.1. Experimental animals

Fertilized eggs (1–2 h post-fertilization) and larvae (2–4 h post-hatching) of gilthead seabream, *S. aurata*, were obtained at the Laboratory of Marine Culture at the Marine and Environmental Sciences Faculty of the University of Cadiz. Embryos (size: 0.8 ± 0.1 mm) and larvae (size: 2 ± 0.2 mm), means ± S.D. ($n = 50$) selected for the test were checked under a stereomicroscope to avoid the use of organisms with obvious disease or alteration (due to handling or culture conditions) that could fake the results of the test.

2.2. Toxicity testing

The experimental design was adapted to the procedure proposed by USEPA (2002) to obtain normalized results for life-cycle studies in early life stages in fish.

Static (i.e. no water replacement) acute toxicity test using different lindane concentrations were performed. Exposure times were 48 h for embryo test (larvae hatching after 48 h) and 96 h for larvae test (larvae start the exogenous feeding due to the endogenous yolk sac reserves are depleted).

The lindane test solutions and the controls of the test were prepared with unpolluted filtered natural seawater (45 μm fibre-glass filter) from Sancti Petri Beach (South Atlantic Spanish Coast). Concentrations of the test were expressed in mg/L lindane.

One liter of each test solution (and controls) was added in 2 L glass vessels at nominal concentrations of 0.1, 0.5, 1.0, 5 and 10 mg/L lindane (2α,3β,4α,5α,6β-hexachlorocyclohexane, γ-isomer, 97%; Sigma–Aldrich Chemie, Germany) for larvae and 0.1, 1 and 10 mg/L for embryos. Two replicates for each concentration including control group were realized. Fifty embryos and fifty larvae were placed in each vessel with continuous slight aeration. Exposure water was analyzed daily for temperature (19 ± 1 °C), dissolved oxygen (95% saturation), pH (8.15 ± 0.06), salinity (39.6 ± 0.1); values were expressed as means ± S.D. The photoperiod during the assay was (12 light/12 h darkness).

Dead and living embryos were counted after 24 and 48 h exposure. In the case of larvae test, dead and living animals were recorded daily throughout the 96 h period. Dead embryos and larvae (deposited in the bottom of the vessels) were removed daily from the test vessels to avoid fungal infection of the living animals. Opaque and submerged eggs were characterized as dead embryos. Larvae were considered dead when immobility, opacity and absence of heartbeat were observed.

The survival, mortality and corrected mortality percent were calculated for each concentration:

$$\%\ \text{survival} = \frac{\text{no. of living embryos/larvae}}{\text{no. of total embryos/larvae exposed}} \times 100$$

$$\%\ \text{mortality} = 100 - \%\ \text{survival}$$

$$\%\ \text{corrected mortality} = \frac{\%\ \text{mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100.$$

2.3. Statistical analysis

Percentages of corrected mortality data were used in the statistical analysis.

The computer model (Probit Program Version 1.5) prepared by the US Environmental Protection Agency was used for the calculation of different LC_p (p = percentage of mortality). This program calculates the mean χ^2 statistic for heterogeneity of results. If tabulated value is significantly greater than the calculated value, the results of the experiments fit the model, and the results are statistically valid. The program also estimates the mean linear regression parameters and uses them to calculate the mean LC_p and associated 95% confidence intervals (USEPA, 2002).

2.4. Organism alterations

A microscopy (Leica Leitz DMRBE) coupled with a digital camera (SONY DKA-C30) was the method employed to observe the morphological alterations in *S. aurata* embryos and larvae exposed to lindane at different concentrations and exposure time.

Table 1
Percent of corrected mortality (median and standard deviation) vs. lindane in embryos test

Concentration (mg/L)	% corrected mortality (mean ± S.D.)	
	24 h	48 h
0 (control)[a]	4 ± 0	6 ± 4
0.1	23.95 ± 1.47	52.12 ± 4.51
1	56.24 ± 5.89	74.46 ± 6.01
10	86.45 ± 7.36	100 ± 0.0

[a] In the case of the controls, data correspond to percent of mortality not the corrected mortality percent.

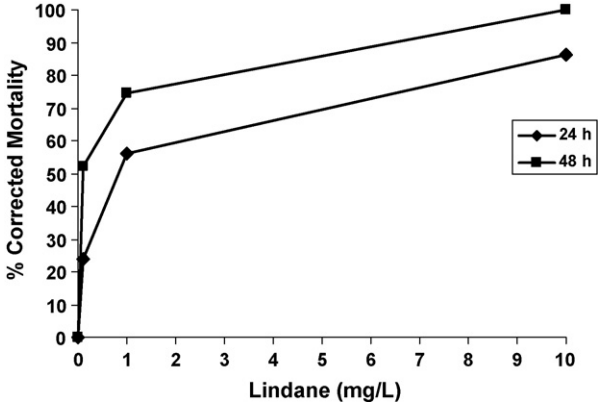

Fig. 1. Relationship between lindane concentration and percentage of corrected mortality in *Sparus auarata* embryos.

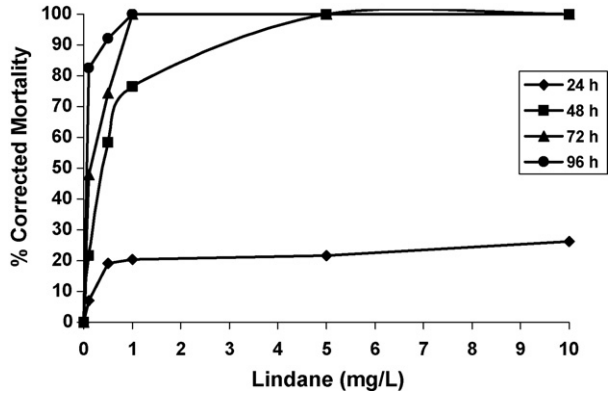

Fig. 2. Relationship between lindane concentration and percentage of corrected mortality in *S. auarata* larvae.

2.5. Histopathology

Larvae from different concentrations of lindane were studied for histopathological manifestations in different tissues. Samples were fixed in 10% formalin and then processed, sectioned, and stained using standard protocol (Roberts, 1989).

3. Results

3.1. Lethal toxicity of the lindane solutions

In embryos and larvae test, control mortality was less than 10%. This percentage was selected previously as quality control of the toxicity test.

Table 2
Estimated LC values and 95% confidence limits for embryos 24 and 48 h exposed to lindane

Toxicity expression	Concentration, mg/L lindane (lower 95%-upper 95%)	
	24 h	48 h
LC_1	0.001 (0.000–0.008)	0.001 (0.000–0.004)
LC_5	0.008 (0.001–0.030)	0.003 (0.000–0.012)
LC_{10}	0.021 (0.003–0.064)	0.006 (0.000–0.023)
LC_{15}	0.041 (0.007–0.105)	0.011 (0.001–0.035)
LC_{50}	0.616 (0.301–1.137)	0.122 (0.041–0.237)
LC_{85}	9.307 (4.209–37.021)	1.329 (0.698–3.628)
LC_{90}	*17.694* (7.083–93.635)	2.338 (1.144–8.269)
LC_{95}	*45.840* (15.009–377.865)	5.402 (2.261–29.475)
LC_{99}	*273.286* (59.462–5339.196)	*25.976* (7.564–342.278)
χ^2 for heterogeneity	0.001	3.454

Tabular $\chi^2 = 3.841$ at 0.05 confidence level. Values in italics correspond with estimated concentrations higher of the upper experimental concentration of the test (>10 mg/L).

The percentages of corrected mortality for embryos and larvae (Tables 1 and 3) are plotted versus lindane concentration to get the response curve (Figs. 1 and 2). The LC_p values (LC_1–LC_{99}) and 95% confidence intervals for different exposure time for embryos and larvae obtained in the Probit analysis are given in Table 2 (embryos) and Table 4 (larvae).

The LC_{50} values showed a gradual decrease with the increase of the exposure time. In general, the increase of the percent mortality was related to both exposure time and lindane concentrations. The highest mortality occurred at 24 h with 10 mg/L of lindane for embryos and for larvae at 48 h with 1 mg/L.

Table 3
Percent of corrected mortality (median and standard deviation) vs. lindane concentrations in larvae test

Concentration (mg/L)	% Corrected mortality (mean ± S.D., $n = 2$)			
	24 h	48 h	72 h	96 h
0 (control)[a]	2 ± 0.0	8 ± 2.0	10 ± 1.0	14 ± 2.0
0.1	7.14 ± 1.44	21.73 ± 3.07	47.82 ± 0.0	82.55 ± 4.93
0.5	22.44 ± 2.88	61.95 ± 19.98	77.17 ± 16.91	93.02 ± 6.57
1	28.57 ± 0.0	81.51 ± 7.68	100 ± 0.0	100 ± 0.0
5	29.59 ± 1.44	100 ± 0.0	100 ± 0.0	100 ± 0.0
10	39.79 ± 1.44	100 ± 0.0	100 ± 0.0	100 ± 0.0

[a] In the case of the controls, data correspond to percent of mortality not the corrected mortality percent.

Table 4
Estimated LC values and 95% confidence limits for larvae 48, 72 and 96 h exposed to lindane

Toxicity expression	Concentration, mg/L lindane (lower 95%-upper 95%)		
	48 h	72 h	96 h
LC_1	0.033 (0.005–0.081)	0.009 (0.001–0.024)	0.000 (0.000–0.005)
LC_5	0.068 (0.015–0.138)	0.020 (0.004–0.044)	0.001 (0.000–0.010)
LC_{10}	0.099 (0.028–0.184)	0.031 (0.008–0.061)	0.002 (0.000–0.015)
LC_{15}	0.129 (0.042–0.224)	0.042 (0.013–0.077)	0.004 (0.000–0.020)
LC_{50}	0.318 (0.216–0.539)	0.143 (0.077–0.212)	0.024 (0.001–0.067)
LC_{85}	1.127 (0.812–1.791)	0.488 (0.338–0.784)	0.154 (0.045–0.310)
LC_{90}	1.457 (1.029–2.569)	0.653 (0.446–1.150)	0.239 (0.102–0.570)
LC_{95}	2.132 (1.421–4.509)	1.006 (0.650–2.093)	0.460 (0.233–2.029)
LC_{99}	4.353 (2.493–13.517)	2.260 (1.255–6.778)	1.565 (0.631–38.295)
χ^2 for heterogeneity	0.572	5.413	2.009

Tabular $\chi^2 = 7.815$ at 0.05 confidence level.

Embryo stage was more sensible than larvae stage. For a specific time, e.g. 48 h, the LC_{50} values of lindane for embryos and larvae were found to be 0.122 (0.048–0.058) and 0.348 (0.216–0.539) mg/L lindane.

3.2. Organism alterations

The study of the malformations have been realised from a qualitative or descriptive (non-quantitative) point of view.

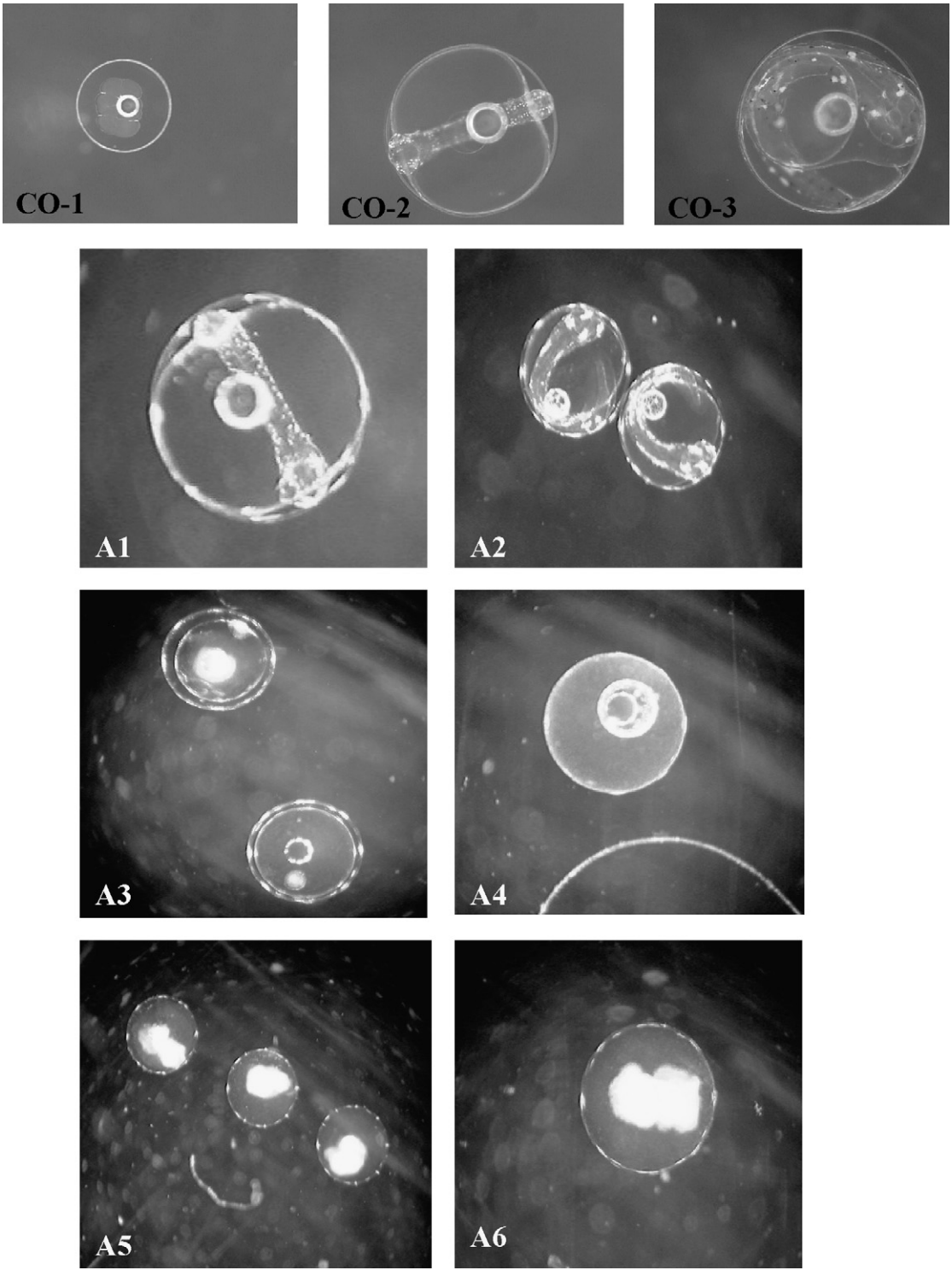

Fig. 3. Morphological alterations in several development stages of *S. aurata* embryos exposed to lindane. CO-1: control 2 h after spawning embryo (25×); CO-2: control 24 h after spawning embryo (26×); CO-3: control 48 h after spawning embryo (28×). A1: embryo exposed to 0.1 mg/L of lindane at 24 h exposure (25×); A2: embryo exposed to 0.1 mg/L of lindane at 48 h exposure (10×); A3: embryo exposed to 1 mg/L of lindane at 24 h exposure (10×); A4: embryo exposed to 10 mg/L of lindane at 24 h exposure (10×); A5: embryo exposed to 1 mg/L of lindane at 48 h exposure (4×); A6: embryo exposed to 10 mg/L of lindane at 48 h exposure (10×).

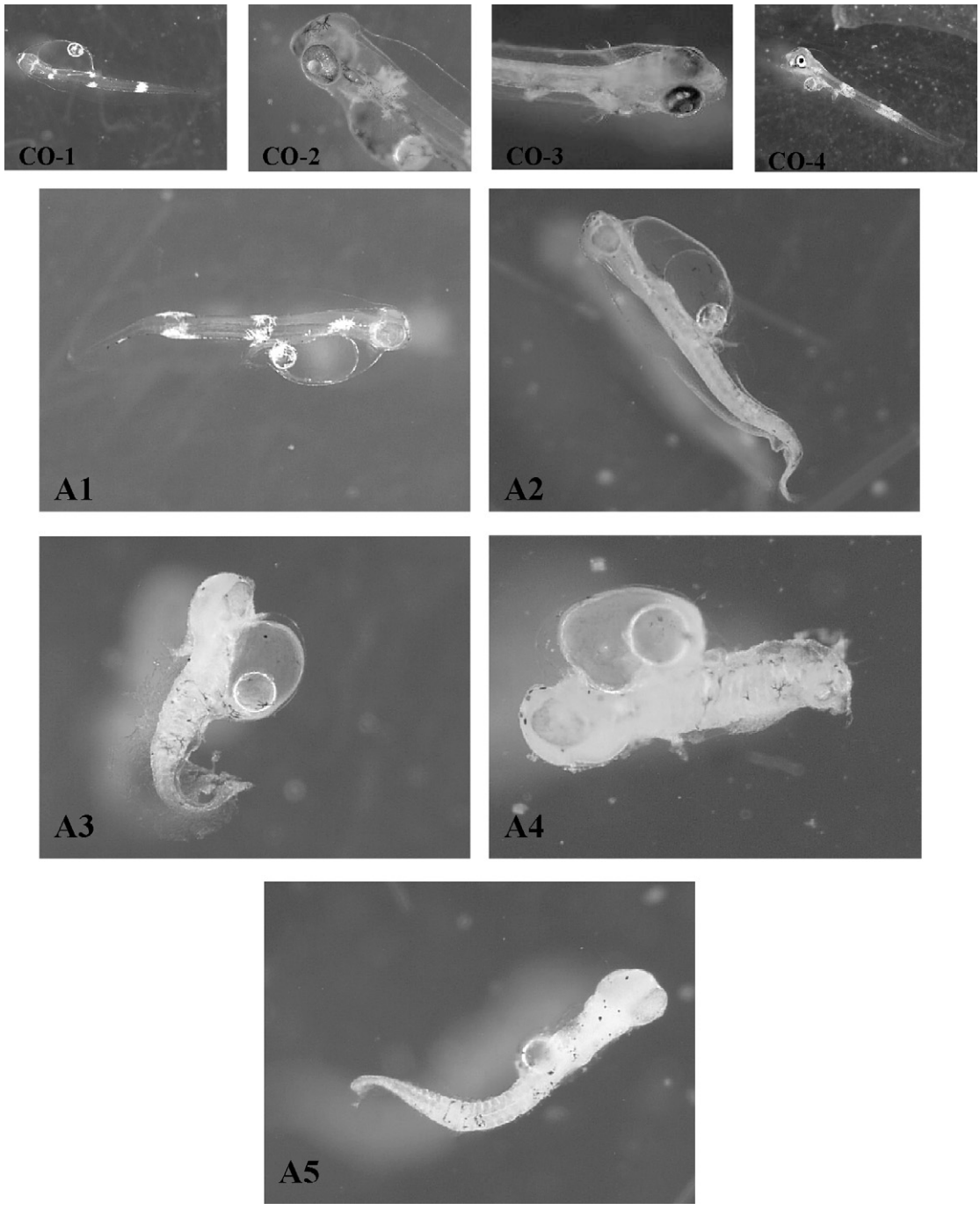

Fig. 4. Morphological alterations on 24 HPH *S. aurata* larvae exposed to lindane. CO: control larvae; CO-1: control larvae 24 HPH; CO-2: control larvae 48 HPH; CO-3: control larvae 72 HPH; CO-4: control larvae 96 HPH. A1: 24 HPH larvae exposed to 0.1 mg/L of lindane (13×); A2: 24 HPH larvae exposed to 0.5 mg/L of lindane (16×); A3: 24 HPH larvae exposed to 1.0 mg/L of lindane (21×); A4: 24 HPH larvae exposed to 5.0 mg/L of lindane; A5: 24 HPH larvae exposed to 10 mg/L of lindane (26×).

The principle deleterious effect observed on chorion was a white coloration (Fig. 3) indicating denaturalization of the proteins of which it is composed (Hampel and Blasco, 2002).

Alterations as depigmentation, weak swimming, trembling, myoskeletal defects, skin opacity and exophthalmia were observed. On 24 h exposure (Fig. 4) an increase of the depigmentation with the increase of the lindane concentrations can be observed. It is observed that an increase of the myoskeletal defects with both time exposure and lindane concentrations (Figs. 5 and 6). Fig. 7 shows two larvae at the lowest lindane concentrations; the malformation grade of these larvae at 96 h exposure is lower than malformation grade of larvae exposed at same concentrations for 24, 48 and 72 h.

3.3. Note on histopathological manifestations

Histopathological manifestations in different tissues of gilthead seabream larvae exposed to several concentra-

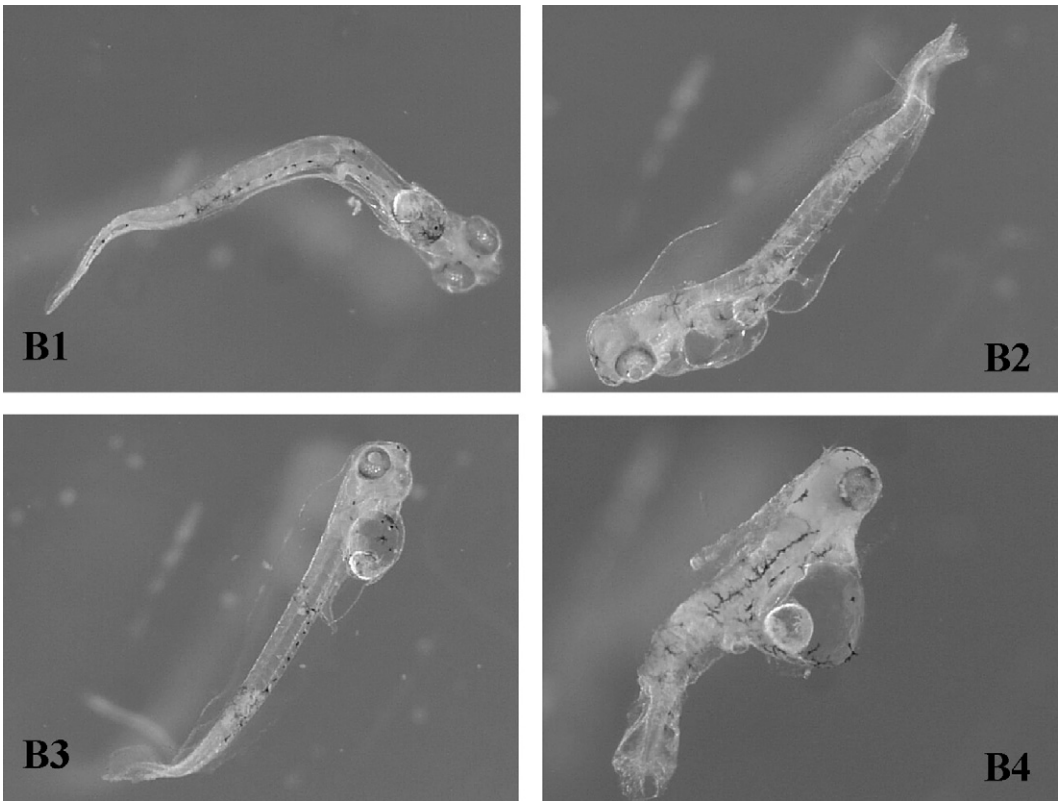

Fig. 5. Morphological alterations on 48 HPH *S. aurata* larvae exposed to lindane. B1: 0.1 mg/L of lindane (15×); B2: 0.5 mg/L of lindane (19×); B3: 1.0 mg/L of lindane (16×); B4: 5.0 mg/L of lindane (25×).

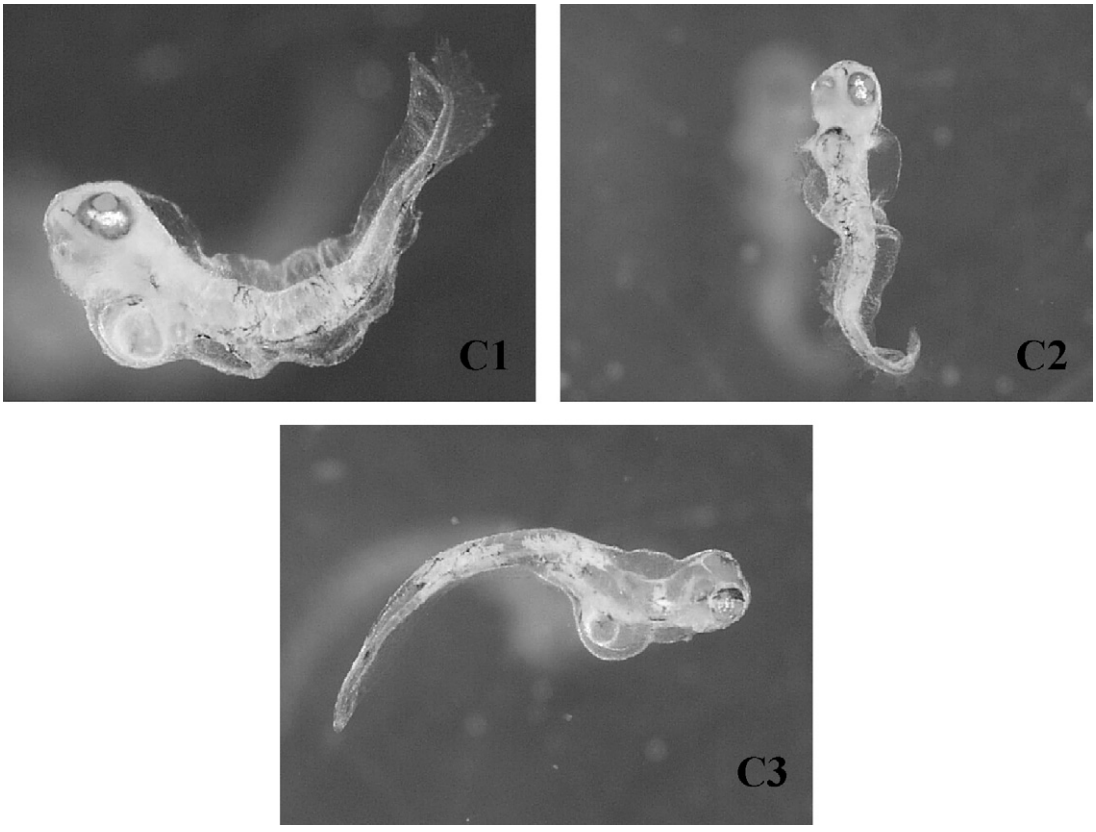

Fig. 6. Morphological alterations on 72 HPH *S. aurata* larvae exposed to lindane. C1: 0.1 mg/L of lindane (25×); C2: 0.5 mg/L of lindane (16×); C3: 1.0 mg/L of lindane (18×).

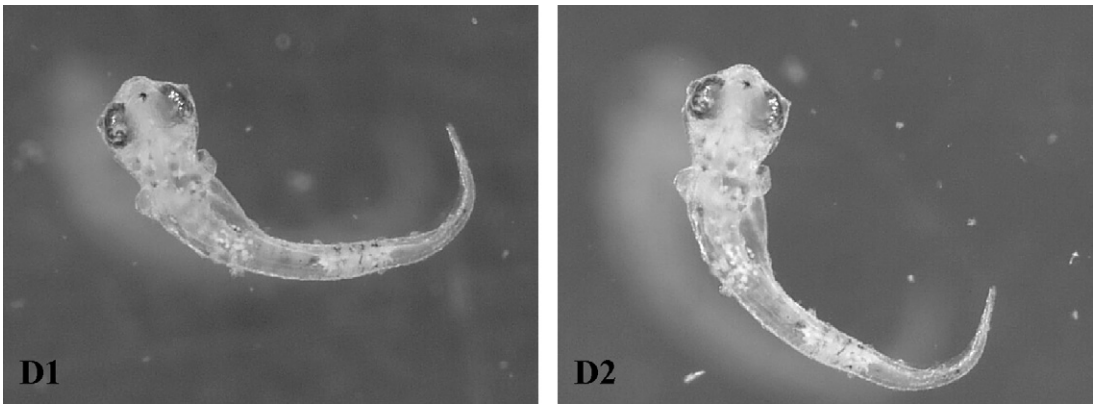

Fig. 7. Morphological alterations on 96 HPH *S. aurata* larvae exposed to lindane. D1: 0.1 mg/L of lindane (16×) and D2: 0.5 mg/L of lindane (20×).

Table 5
Digestive tissue changes of *S. aurata* larvae following exposure to different lindane concentrations

Lesions	Time exposure (h)	Lindane concentration (mg/L)		
		0.1	1	10
Hypertrophy	24	+	+	++
	96	+	++	+++
Atrophy	24	+	+	++
	96	+	++	+++
Epithelium desquamation	24	++	++	+++
	96	++	+++	+++
Glandular alteration	24	–	–	–
	96	–	+	++
Picnosis	24	+	+	+
	96	+	+	++
Necrosis	24	+	+	+
	96	+	++	++

The number of plus symbols is proportional to the assessed damage. Lesion grade: (–) absent; (+) light; (++) moderated; (+++) high.

tions of lindane at 24 and 96 h exposure are showed in Tables 5–7.

We can observe the greater alteration in the digestive tissue (Table 5). At 10 mg/L lindane at 24 h exposure, the mucous cells present a severe alteration due to the hypertrophy and desquamation. The greater hepatic alterations were observed at 1 mg/L of lindane with a total disorganization of the hepatic parenchyma. The injury grade of the renal alterations was not high, but a great cellular disorganization in the kidney hematopoietic tissue

Table 6
Hepatic tissue changes of *S. aurata* larvae following exposure to different lindane concentrations

Lesions	Time exposure (h)	Lindane concentration (mg/L)		
		0.1	1	10
Necrosis	24	–	–	–
	96	+	+	++
Cellular disorganization	24	+	+	++
	96	+	+++	+++

The number of plus symbols is proportional to the assessed damage. Lesion grade: (–) absent; (+) light; (++) moderated; (+++) high.

Table 7
Renal tissue changes of *S. aurata* larvae following exposure to different lindane concentrations

Lesions	Time exposure (h)	Lindane concentrations (mg/L)		
		0.1	1	10
Hematopoietic disorganization	24	–	–	–
	96	+	+	+++
Necrosis	24	–	–	–
	96	–	+	++
Picnosis	24	–	–	–
	96	–	–	+

The number of plus symbols is proportional to the assessed damage. Lesion grade: (–) absent; (+) light; (++) moderated; (+++) high.

is observed in larvae exposed to 10 mg/L (Table 7); the alterations in this tissue have been practically nonexistent to low concentrations.

3.4. Toxicity results and regional water quality criteria of lindane

Andalusia is a region located in the south of Spain between the Atlantic Ocean and the Mediterranean Sea. The regional government of Andalusia has approved the quality objectives (WQO) in seawater (including estuaries, bays and any other enclosure waters as hatchery zones of this fish) of 20 ng/L hexachlorociclohexane (Order 14.02.1997). This WQO is equal to the current objective in the European Unión for the protection of aquatic life in estuaries and territorial seawaters (Directive 84/491/EEC). The toxic concentrations obtained in this work demonstrate than the WQO protects the early stages of *S. aurata*.

4. Discussion

The values obtained in the toxicity test suggest that low levels of these pollutants in the environment induce the presence of alterations and lethal effects in the organisms.

For the protection of aquatic life, the WEF (1992) established numerical criteria for several pollutants and stipulated 0.099×10^{-3} mg/L as a toxic value for lindane dissolved in freshwater and 0.16×10^{-3} mg/L in seawater, but in toxicity

tests with different fish species, the LC_{50} values of organic chemicals can differ, very often, by a factor of more than 10 units (Vitozzi and De Angelis, 1991). Explanation for this phenomenon could be: differences in rates of absorption, distribution in the organism, penetration of organs, metabolism, detoxification, target site, excretion, half-lives of the organism and/or genetic variations. Nevertheless, in studies of aquatic toxicology, the development of predictive models for extrapolation to aquatic environment from one fish species to another is necessary (Cairns and Mount, 1990) producing an intensive research (Barnthouse et al., 1990).

Several reports about physiological and histological effects of pesticides corroborate the lindane toxicity and the variation of the LC_{50} values between different fish species. McDonald (1994) studied the acute toxicity for different fish species obtaining the following results: *Cyprinus carpio* 96-h LC_{50}: 0.09 mg/L, *Fundulus heteroclitus* 96-h LC_{50}: 0.06 mg/L, *Mugil cephalus* 96-h LC_{50}: 0.066 mg/L, *Perca fluviatilis* 96-h LC_{50}: 0.068 mg/L). A 96-h LC_{50} of 0.049 mg/L was observed by Hermens and Leeuwangh (1982) for guppy. Ensenbach and Nagel (1995) observed on larvae of zebrafish (*Brachydanio rerio*) exposed to lindane a 48-h LC_{50} of 0.14 mg/L and a 96-h LC_{50} of 0.11 mg/L.

Although we have diverse information on the toxicity of the lindane in adult stages of fish, we do not have too much information on the toxicity of this compound in early life stages of fish. Despite the sensitivity of *S. aurata*, it was found to have fallen within the range of other fish species reported in literature.

Lindane is efficiently absorbed across the skin, with a documented 9.3% dermal absorption rate. Absorption across the skin as well as in the gut is enhanced by the presence of fat and fat solvents. Although lindane is not highly volatile, pesticide-laden aerosol or dust particles trapped in respiratory mucous and subsequently swallowed may lead to significant absorption in the gut (Reigart, 1999). Following absorption, lindane is partially dechlorinated and oxidized, promptly yielding a series of conjugated chlorophenols and other oxidation products in the urine. Excretion of lindane occurs within a few days, primarily, through the feces. While exposure to most organochlorines results in significant storage of the unchanged parent compound in fat tissue, the rapid metabolic breakdown of lindane reduces the likelihood that it will be detected in body fat and blood (Reigart, 1999).

The greater mortality of embryos in this experiment can be due to the chorion of fish embryos that is considered to be easily permeable to ions (Alderdice, 1988).

The property of the chorion makes possible the maintenance of a hypo-osmotic environment around the embryo. An active regulation appears after gastrulation and is fully developed by yolk plug closure and contributes to keep the egg hypoosmotic. The deleterious effects of lindane on chorion could affect the development of the embryo. This hypothesis is supported by the white coloration observed in the chorion (Fig. 2), indicating denaturalization of the proteins of which it is composed (Hampel and Blasco, 2002).

With respect to distribution in the organism and target site, lindane is a central nervous system stimulant with symptoms usually developing within 1 h (EXTOXNET, 1996). Lindane is a very lipophilic substance like the picrotoxine, inhibiting the postsináptico receptor for the inhibition of the neurotransmitter gamma-aminobutyric acid (GABA). The connection between the GABA and their receptor, called GABA-receptor A, stimulate the ion entrance Cl^- that hyperpolarizes the cell and makes it more resistant to the depolarisation. This way, this insecticide promotes the excitotoxity blocking the stimulation of ions Cl^- by the GABA entrance. This fact can explain the appearance and increase of the myoskeletal defects with both time exposure and lindane concentrations (Figs. 5 and 6). Ensenbach and Nagel (1995) observed two types of abnormalities on zebrafish (*B. rerio*) larvae exposed to lindane: skeletal deformations and edema. These deformations were also found in control groups. The affected animals displayed little activity, and a lot of them remained on the bottom of the aquaria. Not one of these larvae survived. Wester and Canton (1986) observed in young medaka (*Oryzias latipes*) a decreased growth at 0.1 mg/L of lindane. The no-observed-effect concentration (NOEC) was 0.032 mg/L. An abnormal behaviour, characterized by loss of buoyancy and balance, with short episodes of uncoordinated hyperactive movements, was observed in the lower concentration groups, 0.056 and 0.10 mg/L.

From a metabolic point of view, it is possible that the larvae metabolic system is not enough developed so the enzymatic system cannot metabolize the pollutants, bioaccumulation is bigger and formation of easy excretable metabolites decrease.

Fig. 7 shows two larvae at lowest lindane concentrations. The malformation grade of these larvae at 96 h exposure is lower than malformation grade in larvae exposed at same concentrations to 24, 48 and 72 h exposure. The explanation for this fact could be related with the lipid content of the organism and the development of an enzymatic system. The survival of larvae at final of the experiment have absorbed the totality of the vitellum, the lipid content in the organism is greater with respect to anterior larva stages. A high lipid content of an aquatic organism represents an advantage because it protects the organism against toxic effects of lipophilic chemicals (Geyer et al., 1994).

With respect to the histopathological response, a similar response in the organisms was observed after an accidental discharge of lindane into the Barbate River (Cádiz, SW Spain). With a content in water of 0.3×10^{-3} mg/L of lindane and a content in *Barbus* sp. (collected from river) $<0.2 \times 10^{-3}$ mg/kg dry weight, the liver of the sampled fishes presented hepatic cells arranged with a strong cytoplasmic vacuolization (steatosis) and a basophilia increased within cytoplasm of some hepatocytes, which had an excentric and pyknotic nucleus. The kidney showed a disintegration of convoluted tubules, large intracytoplasmic vacuoles in epithelial cells of these tubules. The histopathological and chemical analyses performed in the study suggested a positive relationship between accidental discharge of pesticide and the occurrence of histological alterations in *Barbus* and another fish species from the river, *Mugil* sp. and *C. carpio* (González de Canales et al., 2003).

We conclude that (1) *S. aurata* is suitable species to test the toxicity of chemicals to marine organism due to the short duration of *S. aurata* embryo–larval assay and its sensitivity. (2) *S. aurata* embryos were more sensible to lindane than larvae. (3)

The test with embryos is highly recommendable because it has an easy procedure, very rapid and presents relevant results. We recommended the toxicity test with early life stages of this fish to develop water quality criteria for these water bodies.

Further work in order to complete these results is the realization of bioassays to determine lindane adsorption by the chorion.

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