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PAPER

Ecological risk assessment of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in marine environment using *Isochrysis galbana*, *Paracentrotus lividus*, *Siriella armata* and *Psetta maxima*

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Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are anthropogenic substances classified as persistent bioaccumulative compounds and are found in various environmental compartments throughout the world, from industrialized regions to remote zones far from areas of production. In this study, we assessed the effects of PFOA and PFOS on early life stages of marine test species belonging to three different trophic levels: one microalga (*Isochrysis galbana*), a primary consumer (*Paracentrotus lividus*) and two secondary consumers (*Siriella armata* and *Psetta maxima*). Acute EC_{50} values for PFOS were 0.11 mg L^{-1} in *P. maxima*, 6.9 mg L^{-1} in *S. armata*, 20 mg L^{-1} in *P. lividus* and 37.5 mg L^{-1} in *I. galbana*. In the case of PFOA, the toxicity was lower but the ranking was the same; 11.9 mg L^{-1} in *P. maxima*, 15.5 mg L^{-1} in *S. armata*, 110 mg L^{-1} in *P. lividus* and 163.6 mg L^{-1} in *I. galbana*. The Predicted No Effect Concentration (PNEC) for PFOS and PFOA in marine water derived from these acute toxicity values are 1.1 μg L^{-1} for PFOS and 119 μg L^{-1} for PFOA. This study established a baseline dataset of toxicity of PFOS and PFOA on saltwater organisms. The data obtained suggest that PFOA pose a minor risk to these organisms through direct exposure. In the perspective of risk assessment, early life stage (ELS) endpoints provide rapid, cost-effective and ecologically relevant information, and links should be sought between these short-term tests and effects of long-term exposures in more realistic scenarios.

1. Introduction

Perfluoroalkyl and polyfluoralkyl substances (PFAS) are a group of emerging contaminants made up of a few hundred chemically and thermally stable compounds, mostly polymers, moderately soluble in water. Due to their unique characteristics, PFAS are present in numerous commercial and industrial applications as active ingredients, impurities, or as degradation products of derivatives.[1] It has been shown that the extreme stability of PFAS makes them practically non-biodegradable and particularly persistent in the environment.[2]

Among PFAS, both perfluorooctanoic acid (PFOA) and its sulfonic acid analog, perfluorooctanesulfonic acid (PFOS), were a particular focus of attention because of their widespread occurrence in the environment.[2] PFOS is a highly persistent, bioaccumulative and moderately toxic substance,[1,3–5] widely used in a variety of consumer and industrial products such as pesticides, stain repellents, cleaning agents, corrosion inhibitors,

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Environmental impact

A number of studies have suggested that perfluoroalkyl and polyfluoralkyl substances (PFAS) are highly persistent and bioaccumulative. PFAS can be detected in all environmental media and biota, including humans, and our toxicological knowledge of PFAS in marine organisms is very limited. Perfluorooctane-sulfonic acid (PFOS) is more toxic than perfluorooctanoic acid (PFOA) for all trophic levels, and the ranking of toxicity of species studies was the same. Among all the organisms tested, turbot *P. maxima* showed the highest sensitivity. For risk estimation the Predicted No Effect Concentration (PNEC) for PFOS and PFOA in marine water derived from these acute toxicity values are 1.1 μg L^{-1} for PFOS and 119 μg L^{-1} for PFOA.

flame retardants and fire-prevention agents, adhesives or fire fighting foams.[6,7] PFOA, used as an additive in synthetic industrial products such as corrosion inhibitors, lubricants or wetting agents, has been shown to bioaccumulate in fish, but probably less than PFOS.[8] Published studies show that PFOA is readily absorbed following ingestion, poorly eliminated, and tends not to be metabolized.[9] Immunotoxicity, developmental and reproductive toxicity were also reported.[10,11]

In the marine environment, PFAS have been detected at concentrations up to 58 ng L^{-1} in onshore waters, and 113 pg L^{-1} in offshore waters.[12] Published papers report the presence of perfluorinated compounds in a wide variety of wildlife species, including marine mammals, fish, birds and shellfish.[13,14] The possibility of adverse ecological effects related to the PFAS in the marine environment is needed for an ecological risk assessment (ERA). For risk estimation the quotient of the compartmental concentrations (PEC) and the concentration below which unacceptable effects on organisms will not occur (predicted no effect concentration, PNEC) is calculated. An ERA is performed in phases or tiers and each tier has a higher cost and complexity and less uncertainty. The magnitude of the undesired effects determines the required level of effort. For the aquatic compartment the lower-tier tests are a set of acute bioassays corresponding to three taxonomic groups of three trophic levels.[15]

The toxicity of PFAS has been extensively studied on freshwater species but the data for saltwater species is scarce.[13,16–18] Few standard test methods for saltwater species have been developed and the available data for marine organisms is in general less abundant. To fill this gap, toxicity tests have been developed for saltwater organisms of ecological relevance: a primary producer (the microalga *Isochrysis galbana*),[19] a primary consumer (the echinoderm *Paracentrotus lividus*)[20] and two secondary consumers (the crustacean *Siriella armata* and the fish *Psetta maxima*).[21,22] The flagellate microalga *Isochrysis galbana* is widely found in the coastal waters and easy to culture. *P. lividus* is a sea urchin that occurs throughout the Mediterranean Sea as well as in the North-Eastern Atlantic,[23] and plays key ecological roles in the general functioning of ecosystems.[24] *S. armata* is a mysid with a distribution along the European coast from the North Sea to the Mediterranean Sea, short life cycle and easy maintenance. Turbot *P. maxima* is a native European species of both ecological and economic importance. Adult mature stocks are available all year round because this species is reared under controlled conditions for aquaculture purposes.

This study aimed to assess the effect of exposure to PFOS and PFOA in four marine organisms belonging to three trophic levels, to estimate the PNEC and to conduct the first step of an ERA of PFOS and PFOA in the marine environment.

2. Material and methods

2.1. Experimental solutions

Solutions of perfluorooctanoic acid ($CF_3(CF_2)_6COOH$) (96% purity Sigma Aldrich St. Louis, MO, EE.UU) and perfluorooctanesulfonic acid ($C_8HF_{17}O_3S$) (98% purity Sigma Aldrich, St. Louis, MO, USA) were obtained by dissolving them in 0.22 μm filtered sea water (FSW) of oceanic characteristics from the Ria of Vigo (NW Iberian Peninsula), mixing, overnight stabilization and dilution of the stocks with FSW. For testing high PFC concentrations, stock solutions were prepared in DMSO and added to the test medium at a final maximum DMSO concentration of 0.01% (v/v), plus one solvent control. Experimental concentrations were chosen on the basis of some preliminary tests for these species (Table 1). Glass vials were used instead of plastic vials due to the organic nature of PFOS and PFOA and there is literature supporting the use of glassware.[25,26] All glass material was soaked in 10% HNO_3 for 24 h and rinsed with acetone and Milli-Q water before the experiments.

2.2 Toxicity testing

Bioassay with microalgae (Isochrysis galbana). The microalga test followed OECD[25] as modified by Pérez *et al.*[19] An algal strain of *Isochrysis galbana* was kindly provided by Estación de Ciencias Mariñas de Toralla (ECIMAT). Cultures of *Isochrysis galbana* were grown in 250 mL Erlenmeyer flasks with auto-claved filtered (0.22 μm) sea water and EDTA-free f/2 culture medium. Flasks were kept in an isothermal room at 20 °C with a 24 h light period (cool daylight lamps Osram L36W/865, emission spectrum range 380–780 nm, light intensity 60 μE m^{-2} s^{-1}). An inoculum culture was previously prepared in a 6 L round-bottom flask with bubbling filtered air 1 day before starting the test in order to reach the exponential growth phase. The experimental dilutions were inoculated at a density of 10 000 cells mL^{-1}, and each dilution and control were performed in triplicate. The samples were manually shaken each day and cell counts were carried out at 0, 24, 48 and 72 h with a Multisizer 3 Coulter Counter particle size analyzer (Beckman-Coulter, Miami, FL, USA), and three measurements from each flask were recorded. The inhibition growth rate was calculated in the interval from 0 to 72 h as described in the OECD guidelines.[27]

Bioassay with sea urchin (Paracentrotus lividus). Sea urchin tests followed the methods of Sáco-Álvarez *et al.*[20] The gametes of *Paracentrotus lividus* were obtained by dissection of a couple of adults, and their maturity (ovum sphericity and sperm mobility) was checked with a microscope. The ova were transferred to a 100 mL graduated cylinder containing sea water, *ca.* 10 μL of the sperm taken from the male gonad were added through a Pasteur pipette, and the mixture was shaken gently to facilitate fertilization. The fertilization rate was determined in quadruplicate in samples of 100 individuals, as the proportion of eggs with a fertilization membrane. Within 30 min, the fertilized eggs were transferred to vials with 10 mL of FSW dosed with the product to be tested. Each vial received 400 eggs and each dose was performed in quadruplicate (the control was performed in quintuplicate). The eggs were incubated in the dark at 20 °C for 48 h, and the larvae were fixed by adding 0.2 mL of 40% buffered formalin. In each vial, the maximum length of 35 individuals was measured using an inverted microscope and Leica QWIN image analysis software version 3.4.0 (Leica Microsystems, Germany). The response was quantified as described in Rial *et al.*[28]

$$Ri = 1 - \frac{\Delta L_i}{\Delta L_0} \quad (1)$$

where ΔL_0 and ΔL_i are the mean length increases in the control and the i^{th} dose, respectively.

Table 1 PFOS and PFOA nominal concentrations (mg L^{-1}) used in toxicity test

Species	Group	PFOS (mg L^{-1})	PFOA (mg L^{-1})
Isochrysis galbana	Prymnesiophyceae	3.75, 7.5, 15, 30, 60	25, 50, 100, 200, 400
Daphnia magna	Cladoceran	5, 10, 20, 35, 50, 75, 100, 200	150, 200, 250, 300, 350, 400, 450, 500, 800
Siriella armata	Mysidacea	1.25, 2.5, 5, 10, 20	0.1, 0.5, 1, 2, 5, 10, 20, 30, 40, 80
Paracentrotus lividus	Echinoidea	0.5, 1, 2, 5, 10, 20	1, 2, 5, 10, 20, 50, 100, 200, 500, 750
Psetta maxima	Teleostei	0.015, 0.030, 0.075, 0.15, 0.3, 0.325, 0.6, 1.2, 2.5, 5	1.5, 3, 5, 10, 12, 24, 100, 200

Bioassay with misidacea (Siriella armata). Mysid tests followed Pérez and Beiras.[21] Swarms of *Siriella armata* were captured in the Ría de Vigo (Galicia, NW Iberian Peninsula) and placed in quarantine facilities at ECIMAT. In the laboratory, the mysids were maintained in 100 L plastic tanks with circulating sand-filtered seawater at a rate of 2 L min^{-1}. The adult stock was fed daily with nauplii or metanauplii of *Artemia salina*, *ad libitum*, and parameters were checked daily (temperature ranged between 17 and 18 °C, salinity between 34.4 and 35.9‰, and oxygen 6 mg L^{-1}).

One day before the start of the test, mature females bearing marsupium embryos in the last stage of development were separated in well-aereated separate tanks. The neonates released within less than 24 h were used in the tests. Incubations were conducted in 20 mL glass vials. A total of twenty individuals were used for each concentration, and, in order to prevent cannibalism among neonates, a single individual per vial was used. Oxygen concentration, pH and salinity were determined at the beginning and at the end of each test. Vials were incubated in an isothermal room at 20 °C and a 16 h light : 8 h dark period for 96 h. Daily neonates were fed between 10–15 nauplii of *Artemia salina*. Mortality was recorded after 96 h.

Bioassay with turbot (Psetta maxima). Turbot tests followed Mhadhbi *et al.*[29] Turbot eggs from a single stock of adults were kindly supplied by a fish hatchery (PESCANOVA Insuiña, Mougás, Galicia, NW Spain). The eggs were transported to the laboratory in portable ice-box plastic bags containing seawater, and maintained in aquaria with running natural seawater (salinity 34‰). Eyed eggs were allowed to acclimatise to laboratory conditions at 14 ± 1 °C (hatchery rearing temperature) before being exposed to the toxins. At 72 h post-fertilization (hpf), the floating fertilized eggs were collected and the non-fertilized eggs at the bottom discarded. The eggs were examined under a dissecting microscope, and those embryos exhibiting normal development that had reached the blastula stage were selected for subsequent experiments. Briefly, 50 normal fertilized eggs were randomly selected and distributed into exposure glass beakers containing 500 mL FSW and spiked with the test solutions. Treatments were incubated per quadruplicate in an isothermal room (18 ± 1 °C), in the dark. Neither food nor aeration was provided during the bioassays. Test conditions are summarized in Table 2.

The effects of the toxicants on turbot embryos and larvae were observed throughout the 6-day exposure period and dead embryos and larvae were removed daily. The number of dead eggs/embryos was recorded 48 h after the start of the experiment (from day 0 to 2). Hatching was defined as the rupture of the egg membrane. Partially and fully hatched larvae were counted as hatched. Sublethal endpoints recorded included embryo malformation and hatching success.

Survival of larvae was recorded every 24 h post hatching (hph) from day 2 to 6 of the experiment. At day 6 of the experiment (96 h old larvae), mortality was identified by a missing heartbeat and a non-detached tail. Each larva was carefully placed in a concave slide filled with clean seawater and observed at ×1.5 magnification using a Nikon SMZ1500 MultiScan stereo microscope (Nikon Corp., Tokyo, Japan) with computer image analysis.

All test conditions are summarized in Table 2.

2.3 Statistical analyses

The dose–response relationships were fitted to the modified Weibull model.[30] Fitting parameters were obtained with the statistical software Statistica 8.0 pack, which was also used to calculate the parametric confidence intervals and model consistency (Students t- and Fisher's F-tests respectively in both cases with $\alpha = 0.05$). The maximum no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were established through ANOVA and Dunnett's post-hoc test, using the SPSS application, version 18.0. Non-parametric tests, Kruskall-Wallis and the Mann-Whitney U, were used when data did not meet the requirements of normality and homoscedasticity. Differences were considered as significant when $P < 0.05$.

3. Results

An increase in the concentration of both PFOS and PFOA within the mg L^{-1} range caused growth inhibition in *I. galbana*, embryogenesis inhibition in *P. lividus*, and a survival decrease in *S. armata* and *P. maxima* tests (Fig. 1). The EC_{50} values and their 95% confidence intervals are summarized in Table 3. In all the studied species, the toxicity of PFOS was between 2 and 100 times higher than that of PFOA, with both compounds showing the same ranking of toxicity among different species. Acute EC_{50} values of PFOS in increasing order were 0.11 mg L^{-1} in *P. maxima*, 6.9 mg L^{-1} in *S. armata*, 20 mg L^{-1} in *P. lividus* and 37.5 mg L^{-1} in *I. galbana*. In the case of PFOA, the EC_{50} values were consistently higher; 11.9 mg L^{-1} in *P. maxima*, 15.4 mg L^{-1} in *S. armata*, 110 mg L^{-1} in *P. lividus* and 163.6 mg L^{-1} in *I. galbana*.

Values of EC_{10}, NOEC and LOEC showed the same ranking of toxicity as EC_{50} values in the case of PFOA. For PFOS, the ranking of EC_{50} values was similar, with higher toxicity for the sea urchin *P. lividus* than for the mysid *S. armata.* Therefore, the sensitivities of the test species for PFOS considering toxicity

Table 2 Summary of test conditions for *Isochrysis galbana*, *Paracentrotus lividus*, *Siriella armata* and *Psetta maxima*

	Isochrysis galbana	*Paracentrotus lividus*	*Siriella armata*	*Psetta maxima*
Test type	Static, no-renewal	Static, no-renewal	Static, no-renewal	Semi-static
Age of test organisms (hours)	72	<0.5	<24	72
T/°C	20	20	20	18
Photoperiod	24 h light	Darkness	16 h light : 8 h darkness	Darkness
Test chamber aeration	No	No	No	No
Nr. organisms per test chamber	10 000 cells mL^{-1}	400	5	50
Nr. replicate chambers per concentration	3	4	4	4
Test solution volume (mL)	200	10	2–4	500
Feeding rate	No feeding	No feeding	10–15 nauplii or metanauplii of *A. salina*	No feeding
Test duration (h)	72	48	96	144
Endpoint	Growth inhibition	Growth inhibition	Mortality	Abnormalities/Mortality

thresholds in increasing order were: *P. maxima* > *P. lividus* > *S. armata* > *I. galbana*.

Both PFOS and PFOA caused the same types of body malformations in the early life stages (ELS) (Table 4). For embryos, the most frequently observed response were alterations in yolk sac: at 16 and 14 occasions for PFOS and PFOA respectively. No rupture of the egg membrane was observed in 13 and 11 cases respectively. In the larval stage, the main abnormalities found were the pericardial edema: 22 and 17 cases for PFOS and PFOA, respectively. The second most significant abnormality recorded were the skeletal deformities with 15 and 10 cases, respectively (Fig. 2).

4. Discussion

Environmental risk assessment may require knowledge of acute and chronic toxicity to different trophic levels of the environmental compartment of concern. However, at the moment little is known about the toxicity of perfluorinated compounds in the marine environment, in particular for PFOA and PFOS.

In this manner, the results obtained in these assays provide acute toxicity data for saltwater organisms which allow us to carry out a comparative study between species of different trophic levels and to assess the potential effects of both compounds in wildlife.

The data (Table 3) show a common trend: PFOS is more toxic than PFOA to all the trophic levels studied, particularly for fish. In both cases, the ranking of toxicity was the same: *P. maxima* > *S. armata* > *P. lividus* > *I. galbana*.

The comparison of our results with previous research is not straightforward, since studies on the effects of PFOS and PFOA to aquatic organisms were carried out mainly in freshwater. For instance, the L(E)C_{50} values for PFOS reported by Beach *et al.*[31] in phytoplankton, mysids and fish ranged from 3.5 to 305 mg L^{-1}. Those values are in moderate agreement with the results reported here for PFOS in *I. galbana*, *P. lividus* and *S. armata* (6.9 to 37.5 mg L^{-1}), but *P. maxima* yielded lower values (0.11 mg L^{-1}).

Acute toxicity of perfluorinated compounds to phytoplankton has been investigated thoroughly and the values found are moderate or low. The EC_{50} values reported in the literature for PFOS were: ≥3.2 mg L^{-1} for *Skeletonema costatum*,[32] 48.2 mg L^{-1} for *Selenastrum capricornutum* and 81.6 mg L^{-1} for *Chlorella vulgaris*.[3] In addition, Latała *et al.*[33] reported higher EC_{50} values for *Chlorella vulgaris* and *Geitlerinema amphibium* exposed to PFOA of 386.5 and 977.2 mg L^{-1}, respectively. The EC_{50}/72 h values obtained here for *I. galbana* (37.5 and 163.6 mg L^{-1} respectively for PFOS and PFOA) are lower than those reported in previous studies and, hence, this species seems to be moderately sensitive to these compounds.

Aquatic invertebrates vary markedly in their sensitivity to PFOS and PFOA. This might be explained by differences in life history and physiological response to pollutants. The EC_{50} of PFOS in the sea urchin embryo-larval test (20 mg L^{-1}) was higher than the values determined by Robertson[34] for *Artemia* nauplii (8.9–9.4 mg L^{-1}) but lower than the EC_{50}/LC_{50} values found for *Daphnia magna*, 63 and 130 mg L^{-1}.[1,3] The NOEC in the sea urchin test (1 mg L^{-1}) was similar to the value determined for

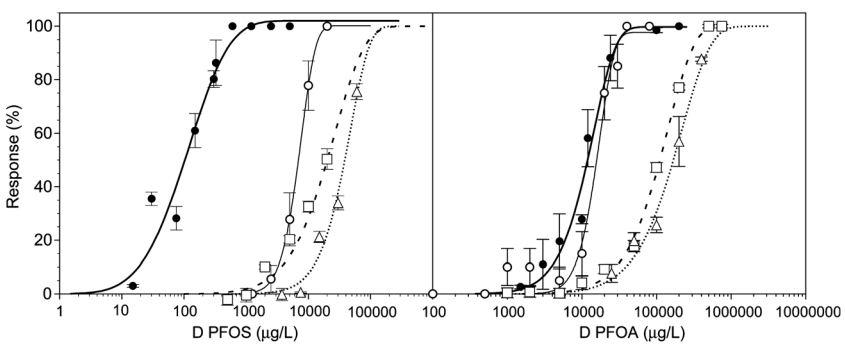

Fig. 1 Percentage of response to PFOS (left) and PFOA (right) for *I. galbana* (···△···), *P. lividus* (–□–), *S. armata* (—○—) and *P. maxima* (—●—). Error bars represent standard error of the mean.

Table 3 Toxicity thresholds (NOEC, LOEC and EC_{10}) and semi-maximum response concentration (EC_{50}) for PFOS and PFOA (mg L^{-1})[a]

Toxicant	Species	NOEC	LOEC	EC_{10}	EC_{50}	EC_{50}/EC_{10}
PFOS	*Isochrysis galbana*	7.5	15	12.2 (8.0–18.5)	37.5 (31.1–45.2)	3.1
	Paracentrotus lividus	1	2	2.6 (1.8–3.5)	20.0 (15.8–25.3)	7.7
	Siriella armata	1.25	2.5	3.2 (3.1–3.3)	6.9 (6.8–7.0)	2.2
	Psetta maxima	0.015	0.03	0.02 (0.01–0.04)	0.11 (0.07–0.16)	5.5
PFOA	*Isochrysis galbana*	25	50	41.6 (25.9–66.6)	163.6 (131.7–203.2)	3.9
	Paracentrotus lividus	10	20	30.7 (25.7–36.8)	110.0 (99.2–121.9)	3.6
	Siriella armata	5	10	7.8 (5.4–11.1)	15.5 (13.0–18.6)	2.0
	Psetta maxima	1.5	3	3.9 (2.4–6.3)	11.9 (9.5–14.9)	3.1

[a] Values are in mg L^{-1}, with 95% confidence intervals in parentheses.

Crassostrea virginica (1.8 mg L^{-1}).[35] Neither a decrease of hatching success nor an increase of morphological abnormalities were found at the highest concentration tested (0.37 mg L^{-1}) in a 16 day embryo-larval test for *Psammechinus miliaris*.[36] The EC_{50} of PFOA for sea urchins, 110 mg L^{-1}, are in line with the values reported for *D. magna*, EC_{50} 181 and 476 mg L^{-1} [1,37] and *M. macrocopa*, 199.5 mg L^{-1}.[37]

Mysids seem to show greater sensitivity to PFAS than algae and sea urchins. Moreover, the NOEC/96 h of PFOA for *S. armata* (5 mg L^{-1}) is less than the chronic NOECs/21d (21 mg L^{-1}) for *D. magna*.[13] On the contrary, the measured toxicity of PFOS to *Siriella armata* (LC_{50}/96h 6.9 mg L^{-1}) is similar but slightly lower than those found in a test conducted with *Mysidopsis bahia* (LC_{50}/96 h 3.5 mg L^{-1}).[38]

The acute toxicity of PFOS to turbot larvae (LC_{50}/96 h 0.11 mg L^{-1}), as expected, was greater than that determined in studies with adult fish, since early ontogenetic stages of fish are regarded as the most sensitive to toxic agents. Therefore, LC_{50}/96 h values for *Oncorhynchus mykiss* acclimatised to salt-water was 13.7 mg PFOS/L[34] and 9.1 mg PFOS/L for *Pimephales promelas*.[39] Furthermore, the lethal toxicity for adult fish is not highly dependent on exposure time, as the LC_{50}/28 d calculated for fathead minnow was 7.2 mg PFOS/L according to Oakes *et al.*[40] Pointing in the same direction as the results found for PFOS, the LC_{50}/96 h of PFOA for turbot larvae, 11.9 mg L^{-1}, is substantially less than the genus mean acute values for *Pimephales promelas* and *Lepomis macrochirus*, 511 and 601 mg L^{-1} respectively.[13]

For PFOS, high sensitivity of the early life-stages of fish has been documented previously[41–44] and this accords with its toxicity to turbot found in the present work. Du *et al.*[41] found that 50 and 250 μg L^{-1} PFOS 30 d treatments reduced body weight and length in zebrafish fry; and Huang *et al.*[42] reported an EC_{50}/120 hpf of 1.12 mg L^{-1} for zebrafish embryos. In the same way, a significant decrease in hatchability and survival, as well as an increase in sublethal malformations of embryos and larvae, were observed here for PFOS and PFOA (Tables 3 and 4). It might be noted that the sensitivity and cost-effectiveness of turbot larval fish test emphasizes the suitability of its routine use in ecotoxicology.

An assessment factor of 100 was chosen on the basis of the lowest short-term $L(E)C_{50}$ in a set comprising at minimum algae, crustaceans and fish to derive a Predicted No Effect Concentration (PNEC) for PFOS and PFOA in marine water.[15] The

Table 4 Morphological abnormalities of turbot embryos larvae exposed to PFOS and PFOA: (A) yolk sac alterations, (B) no rupture of the egg membrane, (C) pericardial edema, (D) skeletal deformities, + indicate number of individuals affected. $n = 200$

Toxin	Concentration [μg L^{-1}]	Embrionary stage (120 hpf)			Larval stage (96 hph)			
		A	B	C	A	B	C	D
PFOS	0.015	—	—	—	—	—	—	+
	0.03	+	—	+	—	—	+	—
	0.075	+	+	—	+	+	++	++
	0.15	+	++	++	+	—	+ +	—
	0.3	+	++	+	++	+	+ ++	+
	0.325	++	—	+	—	—	+++	++
	0.6	++	++	—	++	+	++ +	++
	1.2	++	+++	—	+++	+	++ +	++
	2.5	+++	+++	+	++++	+	++++	++
	5	+++	—	—		—	++++	++
PFOA	1.5	—	+	—	—	++	—	—
	3	+	—	—	—	—	+	+
	5	+	+	++	+	—	+ +	++
	10	++	++	+	++	—	+ +	+
	12	++	—	—	—	—	++	—
	24	++	++	+	+	+	+++	+
	100	+++	++	—	++	+	++ +	++
	200	+++	+++	+++	++	+	++++	+++
Control		+	+	—	+	—	+	+

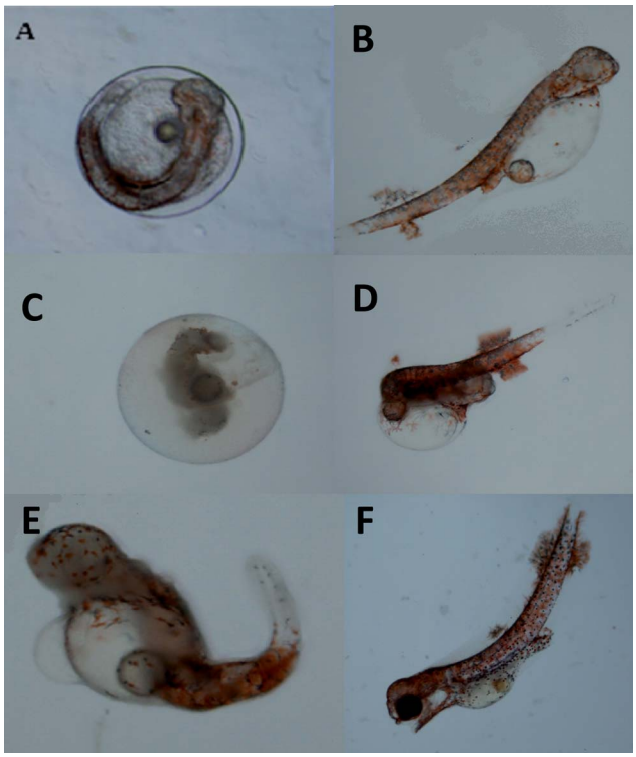

Fig. 2 Morphological abnormalities of turbot embryos larvae exposed to PFOS and PFOA: (A) Normal embryo, (B) Normal larva, (C) no rupture of the egg membrane, (D) yolk sac alterations, (E) pericardial edema, (F) skeletal deformities.

estimated PNECs are 1.1 μg L^{-1} for PFOS and 119 μg L^{-1} for PFOA, a little more conservative than the criteria continuous concentration (CCC) proposed by Giesy *et al.*[13] (5.1 μg PFOS/L and 2.2 mg PFOA/L) for freshwater environments. In order to estimate the level of risk associated with the occurrence of PFAS, the environmental concentrations of those substances were compared with the PNECs. The concentrations of PFOS in coastal and ocean environments are low (Table 5). The PNEC for PFOA 119 μg L^{-1}, is much higher than the range of concentrations found in coastal, 0.076–192 ng L^{-1}, and oceanic waters, 0.015–0.439 ng L^{-1}.[12] However, PFOS levels in effluents of wastewater treatment plants are high and the corresponding hypothetical risk quotient (PEC/PNEC) would take values greater than 1 (Table 5). The concentration of PFAS in municipal wastewater treatment plant effluents is conditioned by domestic (cleaning and care of surface-treated products) and industry use, the contribution of the industry being more important.[45] Likewise, Bossi *et al.*[46] found that the range of PFOS concentrations in effluents ranged from <1.5 to 1115 ng L^{-1} for four industrial plants and from <1.5 to 18.1 ng L^{-1} for six municipal wastewater treatment plants.

The present findings suggest that, although toxic effects of PFOS might occur at μg L^{-1} concentrations, such water concentrations are only found in effluents of municipal wastewater treatment plants and industrial plants.

On the other hand, it is worth noting that high concentrations of PFOS have been detected in fish. For instance, PFOS found in the liver was up to 7760 μg kg^{-1} wet weight for plaice (*Pleuronectes platessa*) and 9031 μg kg^{-1} wet weight for eels (*Anguilla anguilla*).[47,48] The bioaccumulation factors based on liver and surface water concentrations derived from field studies varied from 1260 to 125 000 and are significantly larger than the bioconcentration factors obtained in the laboratory, 484 to 4300.31 It has been pointed out that uptake through water and diet may be a relevant exposure route for PFAS such as PFOS and PFOA. It is clear that more studies are needed to shed light upon whether PFOS concentrations in fish might have toxic effects, as the proposed critical body residue, 87 mg PFOS/kg,[13,31] was derived from lethal endpoints and an assessment factor was used neither to extrapolate from lethal to sublethal effects and from laboratory to field conditions, nor to account for interspecific differences.

5. Conclusions

In all the species studied, perfluorooctanesulfonic acid (PFOS) is more toxic than perfluorooctanoic acid (PFOA), and for both toxicants, the ranking of toxicity was the same. Among all the organisms tested, turbot *P. maxima* showed higher sensitivity, while *I. galbana* was the most resistant.

Up to now, little information has been published about the toxicity of perfluorinated compounds in saltwater, such as those used in our study. The results of this research makes it possible to compare the sensitivity of different trophic levels to perfluorinated compounds as well as to assess the potential effects of PFOS and PFOA on marine ecosystems. Such information could improve the scientific basis for PFAS control but perhaps also provide important information for adequate priority setting of environmental remedial activities of polluted sites. However, acute toxicity tests can only be a first step for the assessment of the environmental risk of these chemicals.

Nevertheless, further detailed studies involving the bioaccumulation and biomagnifications potential, reproduction and maternal transfer effects, fate and behavior of these contaminants are deemed important for the future improvement of the risk assessment.

Table 5 Hypothetical maximum risk quotients (RQ) for PFOS in seawater[a]

Medium	Range of PFOS levels (ng L^{-1})	RQ
Costal waters	0.008–57.7[b]	0.1
Oceanic water	0.001–0.078[b]	0.0001
Municipal wastewater treatment plant effluent	41–5290[c]	4.8

[a] Bold values indicate a risk to this species. [b] Yamashita *et al.* 2005.[12] [c] OECD.[26]

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