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Marine environmental risk assessment and acute water quality criterion for pentachlorophenol in coastal waters

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Abstract
Pentachlorophenol (PCP) is a organochlorine biocide that, unlike most other organochlorines, is still in use as timber preservative. Its water solubility, high toxicity, bioaccumulation potential, and the concentrations reported in estuarine waters (up to 0.1 µg L^{-1}) indicate it may pose a risk in coastal environments. Aquatic environrmental regulations are commonly based on standard freshwater organisms that may not represent the sensitivity of marine species. The present study consists of a water quality criteira reevalutation of PCP in coastal waters based on toxicity tests conducted recording sensitive endpoints of marine species representative of coastal ecosystems, following QA/QC standard procedures. The toxicity thresholds (EC_{10}) found were 4.69 µg L^{-1} for *Paracentrotus lividus* sea-urchin embryos, 6.47 µg L^{-1} for *Mytilus galloprovincialis* mussel larvae, and 78.4 µg L^{-1} for *Isochrysis galbana* cells. Therefore, there is only one order of magnitude between the predicted no-effect concentration (PNEC) for early life stages of bivalves and echinoderms and the maximum concentrations actually recorded in coastal water, which yields a remarkable risk quotient for PCP in these highly productive marine habitats. In addition, we have reviewed the ecotoxicological data on PCP toxicity on marine species representative of the main systematic groups, from algae to chordates, and derived a probabilistic acute saltwater quality criterion of 2.66 µg L^{-1}, intended to protect 95% of the marine species. Lack of adequate protection for marine ecosystems in some current PCP national guidelines has been identified.

Keywords Water quality criteria · Species sensitivity distribution · Early life stages · Marine species · Acute toxicity · Pentachlorophenol

Introduction

Pentachlorophenol (PCP) is a relatively soluble organochlorine biocide that, unlike most other organochlorines, is still in use and widely applied as timber preservative, pesticide and disinfectant. Polychlorinated phenols can be also generated during kraft pulp mill operations when chlorine in the bleaching step reacts with natural phenolic compounds (Newman 2017). PCP interferes with normal reproductive and endocrine function in vertebrates, and PCP metabolites showed genotoxic and mutagenic properties, reviewed by Goodman (2001). Its high toxicity, comparatively high water solubility, bioaccumulation potential in marine organisms with bioconcentration factor (BCF) values between 100 and 1000, and the concentrations reported in estuarine waters (up to 0.1 µg L^{-1}; Muir and Edulijee 1999) indicate it may also pose a risk in coastal environments.

Probabilistic environmental quality criteria obtained from species sensitivity distributions (SSD) allow the protection of a given percentage of the species occurring in an ecosystem (e.g. 95%) with a known confidence level (Aldenberg and Slob 1993). This approach was frequently used in order to derive scientifically sound water quality criteria (WQC) (ANZECC 2000; CCME 2007; Durán and Beiras 2013; Durán and Beiras 2017; EC 2011; OECD 1995; US-EPA 1985). The derivation of probabilistic WQC demands the use of toxicity data for a variety of taxa representative of the communities of interest, covering a wide range of phylogenetic and physiological variability (van Straalen and Denneman 1989). Most standard biological models used in experimental aquatic toxicology are freshwater organisms such as daphnia or salmonid fish. Durán and Beiras (2013) demonstrated that for several trace

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metals the maximum admissible concentrations reflected in the applicable legislation were above the toxicity thresholds for early life stages of saltwater species with high commercial value, evidencing the need to improve the regulations by taking into account toxicity data from marine species.

The objective of the present work is to derive acute water quality criteria based on ecotoxicological information with representative marine species, which provide a known degree of protection for coastal species, using an alternative method to the classical deterministic approach. With this aim, toxicity tests with *Isochrysis galbana* microalgae and early life stages of the mussel *Mytilus galloprovincialis*, and the sea-urchin *Paracentrotus lividus* were conducted in our laboratory. After a broad literature survey, the data set was completed to include species representative of all major marine taxa: algae, annelids, molluscs, crustaceans, echinoderms and chordates. In order to maximize sensitivity and thus protective value of the resulting criterion, preference was given to sublethal endpoints and early life stages. The derived WQC will be compared to national and international criteria and the degree of protection offered by them will be discussed.

Material and methods

Toxicity data set

Due to the large variability in the sensitivity to a chemical among the different species of an ecosystem, US-EPA (1985) requires the use of 8 different families for the derivation of saltwater criteria, while EC (2011) demands at least 10 species covering a minimum of 8 taxonomic groups. The current study used 20 endpoints obtained from 19 species including at least one representative of each of the main taxonomic groups of marine organisms: algae, annelids, mollusks, crustaceans, echinoderms and chordates. In order to maximize sensitivity, and thus protective value of the WQC, early life stages (embryos, larvae, neonates), sublethal endpoints (algal population growth, size growth, reproduction, embryogenesis and larval development) and, when available, long-term exposures were chosen. Because of lack of sensitivity, data on acute adult mortality were not considered.

Toxicity tests methods

Toxicity tests were carried out under strict quality assurance/quality control following internationally adopted standard methods. Growth inhibition tests with *Isochrysis galbana* microalgae followed ISO (2006) and OECD (2006). A non-axenic culture of *I. galbana* was grown in 500 mL flasks in a f/2 medium, and kept in an incubator at 20 °C with 24 h light cycle intensity 60 mE m^{-2} s^{-1} using cool daylight lamps (Osram L36W/865; emission spectrum range 380–780 nm). When reaching the exponential growth phase, an intermediate experimental culture, inoculated with the previous culture (density 7000 cells mL^{-1}), was carried out in 5 L Erlenmeyer flask. Experimental solutions were added to 250 mL borosilicate Erlenmeyer flasks in triplicate and three additional flasks as control cultures. No agitation was provided during incubation. Cell density was measured at the beginning and after the 72 h of exposure with a Z2 Coulter Counter particle size analyzer (Beckman-Coulter Particle Count and size analyzer USA). Growth rate (GR) was calculated as:

$$\mathrm{GR}\left(\mathrm{day}^{-1}\right)=\frac{[\ln(\text{final number cells})]-[\ln(\text{initial number cells})]}{3}.$$

Acceptability criteria for the test were 16-fold increase in cell density of controls in 72 h, and a coefficient of variation among replicates not exceeding 7% (ISO 2006).

Mature mussels (*M. galloprovincialis*) and sea-urchins (*P. lividus*) were collected by scuba divers during the natural spawning season in the outer part of Ría de Vigo (NW Iberian Peninsula). Sea-urchins were maintained in laboratory conditions with circulating sand-filtered seawater at a rate of ca. 9 L min^{-1}, and fed two times per week with macroalgae. Water temperature was 18 ± 2 °C, salinity 34 ± 2 psu and oxygen >5 mg L^{-1}. According to the methods described by Beiras and Bellas (2008) for mussel and Beiras et al. (2012) for sea-urchins, mussel gametes were obtained by thermal induction of spawning, while sea-urchin gametes were obtained by dissection of ripe adults. Mature oocytes of sea-urchins and mussels were transferred to 50 mL measuring cylinders and sperm were added, shaken gently to facilitate fertilization. Fertilized eggs were transferred before the first cleavage into glass vials with airtight teflon-lined caps containing 10 mL of the experimental solutions, at a density of 40 per mL for both species. Four replicates per treatment plus controls were carried out. After 48 h incubation at 20 °C in the dark, vials were fixed with four drops of 40% formalin for ulterior observation under an inverted microscope (Leica DMI 4000B). The endpoints measured were percentage of normal larvae in mussels (n = 100 per vial), and length (maximum linear dimension) in sea-urchins (n = 35 per vial). Mussel larvae were considered abnormal when they did not reach veliger stage, showed irregular shape, convex hinge, and/or protruding mantle (His et al. 1997). Length recordings were made using Leica QWIN image analysis software version 3.4.0 (Leica Microsystems, Germany). Acceptability criteria were percentage of fertilized eggs >98% and size increase in controls >253 µm (Saco-Álvarez et al. 2010) for the sea-urchin test, and control normality > 75% for the mussel test.

Statistical methods

Statistical analyses were conducted using IBM SPSS statistics version 22.0 and DataFit version 9.0 software. *I. galbana* cell growth, larval normality for mussels and larval size for sea urchin were the endpoints analyzed. All data were corrected by the mean control response. Normal distribution and homoscedasticity of the data was checked using the Shapiro-Wilk's and Levene's tests respectively. When significant differences ($p < 0.05$) among groups were found using ANOVA then each treatment was compared to the control using Dunnett's post hoc test to calculate the highest no-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). Non-parametric tests, Kruskall-Wallis and Mann Whitney U were used when data did not meet the requirements for parametric tests. The LC_{50} and LC_{10} values and their 95% confidence intervals were calculated by fitting the data to a modified Weibull dose-response model (Murado et al. 2002), by minimization of the sum of quadratic differences between experimental and model-predicted values using the nonlinear least-squares method provided by the macro Solver of the Microsoft Excel spreadsheet.

The SSD curve for PCF were obtained by fitting for each chemical the cumulative distribution of the toxicity thresholds (TT) obtained for the different species and life stages to a log-logistic model (van Straalen and Denneman 1989) described by the equation:

$$Cp = 1 - \frac{1}{1 + e^{\frac{\log TT - \log a}{b}}},$$

where *Cp* is the cumulative probability of the TT, and *a* and *b* are fitting parameters. The value of *a* equals the TT value for a cumulative probability of 0.5, and the value of *b* is inversely related to the slope of the curve. Non-linear fitting was performed using SigmaPlot (version 10.0) statistical software.

Due to the well-known weaknesses of the NOEC/LOEC approach, such as dependence on experimental design and statistical power (OECD 1998; Reiley et al. 2003; Vighi et al. 2003), EC_{10} were preferred for estimation of the TT. In the cases where EC_{10} data were not available, the TT was estimated using $EC_{50}/3$, or LOEC, based on the mean ratios between EC_{10} and the remaining toxicity parameters obtained from the toxicity data base generated in our laboratory for >60 compounds tested with marine species ($EC_{50}/EC_{10} = 3.3$, $n = 202$; $EC_{10}/LOEC = 1.1$, $n = 137$).

Following previous consensus (EC 2011; US-EPA 1985; van Straalen and Denneman 1989), the 5th percentile (HC_5) of the TT distribution will be used, and in order to take into account the probability that the actual value was lower than the estimate, which would cause under-protection, the WQC will be defined as the lower end of the 95% confidence intervals for the HC_5 (Aldenberg and Slob 1993; Durán and Beiras 2013; Durán and Beiras 2017; EC 2011; Smith and Cairns 1993). Therefore, the WQC should protect 95% of the species with a 95% certainty.

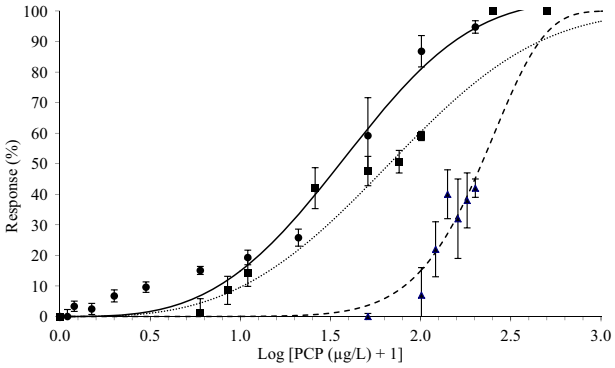

Fig. 1 Percentage of control-corrected response (R): population growth rate inhibition for *I. galbana* (triangles), abnormal larvae for *M. galloprovincialis* (squares) and growth inhibition of *P. lividus* larvae (circles) as a function of PCP concentration

Risk quotient (RQ) was calculated according to the expression (Tato et al. 2017):

RQ=MEC/NOEC,where *MEC* is the measured environmental concentration and *NOEC* is the lowest no observed adverse effects concentration of the most sensitive organism tested.

Results and discussion

The dose:response curves for the three species tested are shown in Fig. 1, and the EC_{50} and EC_{10} values obtained from the curve equations, including their 95% confidence intervals, as long as the NOEC/LOEC values determined by the Dunnett's test are shown in Table 1. NOEC for the most sensitive species tested was 1 µg L^{-1}. Considering reported maximum environmental concentrations of 0.1 µg L^{-1} (Muir and Eduljee 1999), this yields a risk quotient, RQ = 0.1, indicating a 'medium' level of risk (Tato et al. 2017).

The TT values of PCP for each marine species are compiled in Table 2, and the corresponding SSD curve adjusted to those values is presented in Fig. 2. The fitting parameters, *a* and *b*, from the log-logistic model were both significant ($p < 0.05$), and the curve adjusted well to the compiled TT values ($R^2 = 0.9797$). Parameter *a* was 24.80 µg L^{-1} (95% CI 24.12–25.47), and it indicates the median TT value for all marine species. According to this parameter PCP acute toxicity to marine organisms is similar to that of chemicals considered as highly toxic, such as mercury, copper, nonylphenol, pyrene or fluoranthene (Durán and Beiras 2013; Durán and Beiras 2017).

Table 1 No observed effect concentration (NOEC), lowest observed effect concentration (LOEC), and effective concentrations reducing a 10 and 50% the response (EC_{10} and EC_{50}, respectively) for PCP

	I. galbana	*M. galloprovincialis*	*P. lividus*
NOEC	100	5	1
LOEC	120	7.5	2
EC_{10}	78.36 (37.82–118.91)	6.47 (0.96–11.98)	4.69 (2.78–6.59)
EC_{50}	214.40 (163.85–264.94)	66.13 (25.78–106.48)	37.25 (29.12–45.39)

All concentrations are μg L^{-1}. The 95% confidence intervals of the effective concentrations are given in brackets

Table 2 Pentachlorophenol toxicity thresholds (TT) for 19 species representative of the main marine taxa

Taxon	Species	Endpoint	Duration	TT	μg L^{-1}	Reference
Microlgae	*Monochrysis sp.*	Population growth (96 h)	Short term	$EC_{50}/3$	66.7	Adema and Vink (1981)
	Skeletonema costatum	Population growth (5 d)	Short term	NOEC	11	Euro-Chlor (1999)
	Isochrysis galbana	Population growth (72 h)	Short term	EC_{10}	78.4	Present study
Annelids	*Ophrytrocha diadema*	Reproduction (30 d)	Long term	NOEC	10	Adema and Vink (1981)
	Platynereis dumerilii	Embryogenesis (48 h)	Short term	$EC_{50}/3$	173	Palau-Casellas and Hutchinson (1998)
Molluscs	*Mytilus galloprovincialis*	Early larval growth (48 h)	Short term	EC_{10}	6.47	Present study
	Crassostrea virginica	Embryogenesis (48 h)	Short term	$EC_{50}/3$	13.3	Borthwick and Schimmel (1978)
	Crassostrea virginica	Larval mortality (12 d)	Long term	$EC_{50}/3$	23.3	Davis and Hidu (1969)
	Crassostrea gigas	Embryogenesis (48 h)	Short term	$EC_{50}/3$	17.0	Woelke (1972)
	Haliotis rufescens	Embryogenesis (48 h)	Short term	$EC_{50}/3$	16.6	Hunt et al. (1996)
Crustaceans	*Echinogammarus marinus*	Growth (56 d)	Long term	NOEC	100	Adema and Vink (1981)
	Siriella armata	Neonate mortality (96 h)	Short term	LC_{10}	90.7	Pérez et al. (2015)
	Palaemon elegans	Larval mortality (96 h)	Short term	$LC_{50}/3$	28	van Dijk et al. (1977)
	Crangon crangon	Larval mortality (96 h)	Short term	$LC_{50}/3$	37	van Dijk et al. (1977)
	Tisbe battagliai	6 day copepodid stage mortality (24 h)	Short term	$LC_{50}/3$	151.81	Smith et al. (1994)
Echinoderms	*Paracentrotus lividus*	Early larval growth (48 h)	Short term	EC_{10}	4.69	Present study
Chordates	*Lagodon rhomboides*	2d larva mortality (96 h)	Short term	$LC_{50}/3$	12.7	Borthwick and Schimmel (1978)
	Pleuronectes platessa	Development from egg (56 d)	Long term	NOEC	10	Adema and Vink (1981)
	Cyprinodon variegatus	Survival and growth (life cycle)	Long term	NOEC	47	Euro-Chlor (1999)
	Sillago japonica	Hatching inhibition (24 h)	Short term	$EC_{50}/3$	46	Onikura et al. (2007)

In order to enhance sensitivity, acute lethal toxicity data with adults was not considered

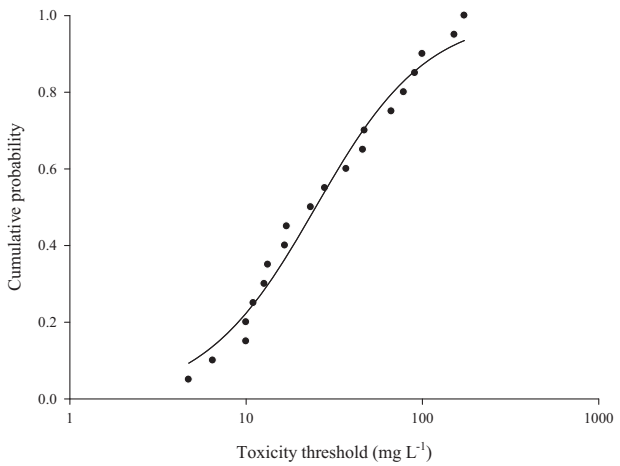

Fig. 2 Distribution of the cumulative probability of toxicity thresholds (TT) for marine species for PCP. Dots represent the individual TT values from Table 2

Parameter *b* was 0.32 (95% CI 0.30–0.33), and it is inversely related to the slope of the curve, and thus provides information on the degree of variability in sensitivity among species. Despite the large taxonomic differences among testing organisms, TT values ranged less than two orders of magnitude, from 4.69 (obtained with the most sensitive species, *P. lividus*) to 173 μg L^{-1} (for the least sensitive species, the annelid *P. dumerilii*). The good fit to a single curve for all species tested and the low value of *b*, points at a universal mechanism of toxicity for PCP, commonly considered as an uncoupler of mitochondrial oxidative phosphorylation. We have previously reported *b* values ranging from 0.23 to 0.37 for non-selective organic toxicants (Durán and Beiras 2017).

The 5th percentile (HC_5) of the SSD, its 95% confidence intervals (CI), the critical value, and the proposed acute WQC obtained from the lower end of the CI, are summarized in Table 3, which reflects also the standards and

Table 3 Percentile 5 (HC_5), 95% confidence intervals (CI), critical value and acute Water Quality Criteria (WQC) obtained in the present study, and other environmental regulations from national and international administrations for marine waters except when otherwise stated

HC_5	CI	Critical value	Acute WQC	2013/39/EU		US EPA		ANZECC	Canada
				Chronic	Acute	Chronic	Acute	95%	
2.90	2.66–3.14	4.73	2.66	0.4	1	7.9	13	22	0.5[a]

All concentrations are $\mu g\ L^{-1}$

[a]Freshwater criteria, not regulated for marine waters

criteria stated in different environmental regulations. The EU set the same standards for continental and marine waters, and we can see that the acute value ($1\ \mu g\ L^{-1}$) is sufficiently protective at the light of the ecotoxicological information here reviewed. Canada implemented a freshwater criterion that seems suitable for marine waters also ($0.5\ \mu g\ L^{-1}$; CCME 2007). In contrast the values adopted by US-EPA ($13\ \mu g\ L^{-1}$; US-EPA 2017) and ANZECC ($22\ \mu g\ L^{-1}$) are not sufficiently protective for marine ecosystems, since they are above the acute WQC here calculated, and even above the TT experimentally demonstrated for bivalve and sea-urchin larvae.

In conclusion, the acute WQC here derived from marine ecotoxicological tests are higher than those imposed by the EU and Canada, which may be slightly too conservative. In contrast, they suggest an insufficient protection of coastal marine ecosystems if US EPA and ANZECC criteria are applied.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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