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The European water-based environmental quality standard for pentachlorophenol is NOT protective of benthic organisms

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Abstract

Risk management of toxic substances is often based on Environmental Quality Standards (EQS) set for the water compartment, assuming they will also protect benthic organisms. In the absence of experimental data, EQS for sediments can be estimated by the equilibrium partitioning approach. The present study investigates whether this approach is protective of benthic organisms against pentachlorophenol (PCP), a legacy contaminant and EU priority substance still used in some parts of the world. Three freshwater species of invertebrates with different life cycles and feeding behaviours (the oligochaetes *Lumbriculus variegatus*, *Tubifex tubifex* and the dipteran insect *Chironomus riparius*) were exposed to PCP spiked sediments (2.10–46.03 mg PCP/kg d.w. plus controls) in laboratory standard tests. Exposure duration was 28 days for *T. tubifex* and *L. variegatus* and 10 and 28 days for *C. riparius*; according to the corresponding OECD guidelines. For each investigated end-point, dose-response data were normalized to the mean control and fitted to a four-parameter log-logistic model for calculating the corresponding EC₅₀ and EC₁₀. The ranges for EC₅₀ and EC₁₀ estimates were 4.39 (*Chironomus riparius*-emergence)–27.50 (*Tubifex tubifex*-cocoon) and 0.30 (*T. tubifex*-young worms)–16.70 (*T. tubifex*-cocoon) mg/kg d.w., respectively. The EC₅₀ and the EC₁₀ values of *L. variegatus* were within these ranges. Following the EU Technical Guidance for deriving EQS, the lowest EC₁₀ value of 0.30 mg/kg (*T. tubifex*-young worms) resulted in a PCP quality standard (QS) for sediments of 30 ng/g, about one fourth of the tentative QS of 119 ng/g estimated by the equilibrium partitioning (EqP) approach. The response of benthic biota to PCP varied across organisms and across end-points for the same organism, so that the use of sediment PCP-QS calculated using the EqP-approach may be under-protective of the most sensitive organisms. Information on the possible effects of PCP on resident organisms must therefore be collected for appropriately managing aquatic systems.

Lowest EC_{10}

EQS 30 ng/g d.w

Highlights

- The ecotoxicity of pentachlorophenol (PCP) to benthic organisms was assessed
- Sensitivity to PCP varies across organisms and end-points
- A PCP quality standard (QS) for sediments is proposed: 30 ng/g dry weight
- The European Union water-based QS may be under protective for benthic organisms

1. Introduction

1.1 Environmental Risk Assessment at the European level

Environmental Risk Assessment (ERA) is a fundamental tool for supporting decision making in the regulatory context. At the European level, ERA is guided by two important regulations: the Water Framework Directive (WFD), adopted in 2000, and the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation which came into force in 2007. Within the WFD, European Union Member States (MS) committed themselves to achieve good ecological and chemical status for all water bodies by 2015; however the objective has not fully met and further updates are due until 2021 and 2027 (EEA, 2015). At the same time, compliance with REACH regulation is required for all chemicals manufactured or imported into the EU, unless specifically exempted. During the REACH process, different companies generate a dossier containing data on physico-chemical characteristics, as well as toxicological and ecotoxicological properties for each substance. The dual processes of dossier and substance evaluation lead to the identification of chemicals that may pose unacceptable hazards to human health and/or the environment; also in connection with the WFD implementation (article 11 of Directive 2013/39/EU).

For risk assessment purposes in aquatic environments, REACH considers the water column and the sediments, which differ in chemical-physical characteristics and resident biota. For substances potentially capable of binding to sediments to a significant extent ($\text{Log } K_{ow}$ or $\text{Log } K_{oc} \geq 3$), the European Chemicals Agency (ECHA) supports REACH registrants to use specific standard guidelines to assess the risk of chemicals in sediments and recommends the use of sediment-dwelling organisms for bioassays (ECHA, 2015). Similarly, the WFD and the Daughter Directive (DD) 2013/39/EU (European Commission, 2000; European Commission, 2013) establish Environmental Quality Standards (EQS) only for the water and biota matrices, but acknowledge the usefulness of sediment-based EQS for some priority substances. MS are allowed to develop and enforce EQS for sediments at the national level provided that such EQS are established through a

transparent procedure and ensure a level of protection equivalent to the EQS for water (or biota) set up at the European Community level.

1.2 Pentachlorophenol

Pentachlorophenol (PCP) is a broad-spectrum pesticide which inhibits the synthesis of ATP (Mäenpää et al., 2008) and free amino acids, disrupting the organisms' energy metabolism, influencing the fecundity of certain invertebrates such as amphipods (Graney and Giesy, 1986). PCP is considered as a priority pollutant under the DD 2013/39/EU (European Commission, 2013). Even though its use has been restricted after the 1980s, it is still widely present in the environment, and it is considered as a chemical pollutant of concern (ATSDR, 2001). It has been used throughout the world for purposes as varied as antimicrobial agent, detergent and wood preservative (Gulcan et al., 2008). In 1996, imports of PCP to the EU amounted to 30 t, out of which 28 were synthesized to 46 t of pentachlorophenyl laurate (PCPL). In 1999, the only producer of PCPL in Europe purchased no more than 20 t of PCP for conversion into PCPL and its production of PCPL for 2000 was less than 30 t. With the Directive 91/173/EEC (European Commission, 1991), the marketing and use of PCP in substances or preparations in a concentration equal to or greater than 0.1 % by mass throughout the European Union has been prohibited, with some exceptions for the use of PCP for the treatment of wood, the impregnation of fibers and textiles, as a synthesizing agent in industrial processes and for the treatment of historical buildings. In other regions of the world (notably in China), PCP production still reached 3,000 t in the early 2000s, mainly for use as a molluscicide (Chen et al., 2016). PCP concentrations in water displayed a downward trend in Northern Europe and any risk posed by PCP to the freshwater environment can largely be attributed to sediments contaminated from historic use (Muir and Eduljee, 1999). Due to its relatively high lipophilicity, PCP sorbs strongly to sediments (Sanchez et al., 2005). However in more recent years, PCP levels show increasing trends in some environmental media and regions (Zheng et al., 2011). PCP levels in sediments samples collected (mainly in China) after the year 1998 range from 0.56 to 200 ng/g, with peaks up to 48,300 ng/g at heavily impacted sites (Table S1). The limited available data (Table

S1) on levels of PCP in freshwater sediments indicate an half-life of 2.7 yrs (Zheng et al., 2011), meaning that PCP can persist in the environment for 1 to 2 decades. According to the estimation of Gabbert et al. (2014) after emissions ceased PCP maintains a “stock pollution effect” posing risks to aquatic ecosystems (Muir and Eduljee, 1999; Xing et al., 2012). PCP appears also in the endocrine disruptor list (Li et al., 2010; Zheng et al., 2012) with yet unpredictable effects on organisms.

Both chronic and acute PCP exposure are medical concerns. Acute inhalation exposures in humans have resulted in neurological, blood, and liver effects, and eye irritation (ATSDR, 2001; US EPA, 1999). Chronic exposure to PCP by inhalation has resulted in effects on the respiratory tract, blood, kidney, liver, immune system, eyes, nose, and skin (ATSDR, 2001; US EPA, 1999). Adverse effects of PCP have been recently reported on fetal growth and birth outcomes (Guo et al., 2016). Oral animal studies have reported increases in liver tumors and two uncommon tumor types. EPA has classified pentachlorophenol as a Group B2, probable human carcinogen (ATSDR, 2001; US EPA, 1999).

With the aim of environmental protection, the WFD has set water-based EQS of 0.4 µg/L and 1 µg/L of PCP as the annual average and the maximum admissible concentration in inland and other surface waters, but nothing is specified for biota and sediments where its concentrations are a matter of ongoing concern (Zheng et al., 2011). As the $K_{p_{susp}}$ of PCP is 1.85–3.72, the trigger criterion to calculate a sediment quality standard is met (European Commission, 2011). The data sheet providing background information on the setting of the EQS for PCP (European Commission, 2005) reports a tentative EQS for sediments of 119 ng PCP/g d.w., calculated using the equilibrium partitioning (EqP) method.

1.3 Ecotoxicity of PCP in sediments

Even if not promptly evident, sediment contamination directly and indirectly affects the water environment reaching in the end human beings. Field observations suggest, and laboratory ecotoxicity assays confirm, that contaminated sediments can negatively affect benthic organisms such as oligochaetes and insect larvae, which occupy crucial positions in the food chain. Therefore

selected species of benthic organism in controlled standard conditions can be used to assess the risk of specific substances through exposures in laboratory. Characterization of PCP ecotoxicological potential is available for several organisms and taxonomic groups, including benthic species (Zhao and Zhang, 2017). However, most studies rely on aqueous exposure and deal with acute ecotoxicity and, to the best of our knowledge, only a handful of studies examined PCP effects via exposure of *Lumbriculus variegatus* (Egeler et al., 2005, Mäenpää et al., 2008, Nikkila et al., 2003) or *Chironomus prasinus* (Sánchez et al., 2005) to contaminated sediments. Furthermore, these previous studies used different methodologies and approaches, which complicates the comparison among different organisms. In order to verify whether sediment-based EQS for PCP derived using the EqP approach actually provide an appropriate degree of protection to benthic organisms, the present study intends to characterize PCP ecotoxicity to sediment-dwelling biota, as a model case study. We carried out long-term toxicity tests using three different benthic organisms, representing major taxonomic groups of freshwater benthic invertebrates with different life cycles and feeding behaviors: two species of oligochaetes (*Lumbriculus variegatus* and *Tubifex tubifex*) and an insect (*Chironomus riparius*). The chironomid non-biting midge *C. riparius* (OECD, 2004) is an epibenthic species, the most commonly tested organism among insects. Among the endobenthic aquatic oligochaetes which ingest particles below the sediment surface, Lumbriculidae has received most attention as toxicity test organisms, even if *T. tubifex* has also been used (Reynoldson et al., 1991; Bettinetti et al., 2003; Pasteris et al., 2003; Bettinetti et al., 2005; Maestre et al., 2007; Kiliç et al., 2011). *L. variegatus* is also the main species recommended for assessing prolonged exposure to sediment-associated contaminants (OECD, 2007). Using both *taxa* can ensure exposure to the test substance via all possible uptake routes. Both *taxa* have a wide tolerance to pH and other environmental variables (Chapman et al. 1982; Havas and Hutchinson 1982).

The main goals of the current study were to 1) generate standard ecotoxicological data of benthic organisms to PCP in sediments; 2) characterize the responses of benthic invertebrates to PCP in artificial, spiked sediments; 3) propose a tentative PCP EQS for freshwater sediments.

2. Materials and methods

2.1. PCP analyses in sediments

Sediment samples (around 5 g) were collected from each test beaker at the beginning of the exposure for all tested concentration and also at the end of the exposure for sediments spiked with 5.56 and 50.00 mg/kg d.w. Sediments were stored at -20 °C and shipped frozen to the chemical laboratory for processing and analysis following Tölgyessy et al. (2009). Ultrasonic solvent (methanol and dichloromethane 9:1) extraction was followed by stir bar sorptive extraction. The extracts were redissolved in water and phenolic compounds were derivatised using acetic acid anhydride. The derivatives were preconcentrated on a stir bar coated with polydimethylsiloxane. PCP was analysed by Gas Chromatography-Mass Spectrometry in EI mode. A chromatograph HP 5890 equipped with a HP-5 Crosslinked 5% PH ME Siloxane column was used. Two mL were injected at 240°C in splitless mode. The oven was programmed from 100°C (2 min) to 210°C (5 min) at 5°C/min. The Mass spectrometer was a HP 5989A. Source and quadrupole temperatures were 200°C and 100°C, respectively. Detection was done at 70 eV in the SIM mode (ions 266, 308 m/z), with quantification by the external standard method. The quantification limits of the analytical method were 0.005 mg/kg d.w.

2.2. Culture of test organisms

The oligochaetes *Tubifex tubifex* (originally coming from the laboratory of Prof. Andrea Pasteris, University of Bologna, Italy) and *Lumbriculus variegatus* (originally supplied from Dr Philippe Egeler, ECT Oekotoxikologie GmbH, Germany) were kept in the dark at 21±2 °C in glass containers (diameter 10.5 cm; height 6 cm) half-filled with sterilized quartz sand and dechlorinated tap water (hardness: 320 mg/L CaCO₃). To avoid overcrowding, 25 adults were bred in each beaker. Every week the sand was washed and the water completely renewed. Animals were fed with frozen spinach thawed at room temperature immediately before use and placed beneath the sand (OECD, 2007).

Chironomus riparius (originally coming from RIZA, now part of the Ministry of Infrastructure and the Environment, The Netherlands) were bred at 21 ± 2 °C under daily (16 hours light: 8 hours dark) photoperiod in 40-L aquaria with control sediment (3 cm deep) as substrate. An 8-cm-deep column of dechlorinated tap water (hardness: 320 mg/L CaCO_3) was maintained over the sand. Once a week cultures were fed with frozen spinach and the water was almost completely renewed (OECD, 2004).

2.3. Experimental set up

Pentachlorophenol (PCP) was obtained by Sigma-Aldrich Chemie GmbH, Steinheim (Germany). In order to spike sediments the procedures recommended in OECD Guideline 225 (OECD, 2007) was followed. Since PCP is poorly soluble in water it was dissolved in acetone at different concentrations. Each test solution (2.44 mL) was mixed with 10 g of quartz sand and the solvent was completely evaporated under a fume hood. The dry sand was then added to formulated sediment for each concentration level. The control sediment was prepared with quartz sand without acetone and the sediment for the solvent control was prepared with the same amount of the solvent as the treated sediments. The spiked sediment was equilibrated with the overlying reconstituted water (OECD, 2007) for 7 days at the test condition temperature. The total organic carbon of sediments was evaluated as Loss on Ignition (LOI) (Dean, 1974) at 550 °C after acidification with HCl for 2 hrs.

A range finding test (range of spiked concentrations 0.064–1000 mg/kg d.w., seven concentrations established using a geometric progression with a common ratio of 5) showed that all adult organisms died when exposed to PCP levels of 40 mg/kg d.w.; while no effects were observed at concentrations ≤ 0.32 mg/kg d.w. Based on these results, the nominal concentrations to test sublethal effects of PCP on oligochaetes and chironomids were: 1.85, 5.56, 16.7, 25.3 and 50 mg/kg d.w.

2.4. Bioassays

One day before the addition of the test organisms, 250 mL glass beakers were filled with 70 g of spiked (or control) wet sediment (approximately 50% water content) and directly mixed with 0.5%

of sediments d.w. of dry powdered stinging nettle for oligochaetes, and 3.5 mL of a 4 g/L water suspension of fish food, corresponding to 14 mg d.w. Tetramin[®] for chironomids; 150 mL of reconstituted water (OECD, 2007) was then gently added.

The contents of the beakers, covered with a plastic Petri dish with a hole for aeration, were allowed to settle in the dark at 21 ± 2 °C. Five replicated beakers were prepared for each concentration, including the control and solvent control. At the start of the test, the overlying water of each beaker was gently aerated for 2 h. Oligochaetes tests were performed in the dark, while chironomid tests were performed with a photoperiod of 16 hours of light (1000 lux) and 8 of dark; during the exposures the overlying water was continually aerated. Every 2 days water was added to beakers, if required, to compensate for evaporation.

Temperature, pH, and dissolved oxygen were measured in all the beakers at days 0 and 28, when ammonium was also determined using a colorimetric test kit (Visocolor, Macherey-Nagel, Germany).

Ecotoxicity test with *T. tubifex* (*Tt* test) was performed according to the guideline set by Reynoldson et al. (1991). At day 0 four sexually mature worms at their first reproductive event (approximately 7 weeks) were randomly transferred to each test beaker (five replicate beakers). At the end of the test, the content of each beaker was sieved through 250 µm mesh. The total number of surviving adults was counted immediately, and cocoons and young worms were preserved in 70% alcohol and then counted under the dissecting microscope.

Test with *L. variegatus* (*Lv* test) was carried out following OECD (2007) using spiked sediments (three replicate beakers). The test started with 10 synchronised worms/beaker. To synchronise the worms they were artificially fragmented with a scalpel and the posterior ends were left to regenerate new heads for approximately 3 weeks before the start of the test. The test organisms are therefore expected to be in a similar physiological state. After 28 days the potential impact of the test compound is evaluated on the total number and biomass (as dry weight) of the organisms.

At the start of *C. riparius* (Crip) test, the overlying water of each beaker (three replicate beakers) was gently aerated for 2 hrs and 10 first instar larvae were transferred to each test beaker. Tests were performed under 16:8 h light:dark photoperiod for 10 d. Every three days organisms were fed with 3.5 mL of Tetramin[®] suspension and the water lost through evaporation was replaced. At the end of the 10 days test, the content of half of the total number of the exposed beakers was sieved through 250 µm mesh. The recovered larvae were counted and after gut purging of 4 hours they were weighed (wet weight). In the remaining beakers (five replicate beakers) every day emerged adults were counted until the end of the exposure which lasted 28 days (at maximum).

2.5. Statistical analyses

The EC₅₀ and EC₁₀ correspond to the concentrations of a substance which affect 50 % and 10 % of the exposed population, respectively. In particular, EC₁₀ can be considered as a suitable analogue for the No Observed Effect Concentration (NOEC) (Beasley et al., 2015); i.e. the highest concentration at which there is no statistically significant difference from the control population for a measured end-point (Van der Hoeven, 2004; De Laender et al., 2013).

Dose-response data were normalized to the mean control for each test (Weimer et al., 2012). A four-parameter log-logistic model was fitted to the dose-response data according to:

$$response = c + \frac{d - c}{1 + \exp\{b[\log(concentration) - \log(EC_{50})]\}}$$

In model (1), EC₅₀ enters as one of the model parameters. The parameters b, c and d represent the (Hill) slope (b) as well as the lower (c) and the upper (d) asymptote of the dose-response curve.

EC₅₀ estimates with 95%-Confidence intervals as well as EC₁₀ estimates with 95%-Confidence intervals were obtained. All calculations and visualizations were performed with the statistical software R version 3.2.3 (Team R Development Core, 2015). The four-parameter log-logistic model was fitted using the R package drc (Ritz and Streibig, 2005). For pairwise comparison of EC₅₀ resp. EC₁₀ estimates between end-points, the ratios of the EC₅₀ resp. EC₁₀ values for different end points was estimated and 95%-Confidence intervals were calculated.

3. Results and discussion

3.1 PCP ecotoxicity to benthic organisms

Measured concentrations (MC) of PCP in test sediments were within 4.8-11.0 % of nominal concentrations and remained stable over time, indicating the absence of major PCP losses due to abiotic factors over test duration (Table 1).

Table 1. Nominal (NC) and measured (MC) concentrations of PCP in sediments (mg/kg d.w.) at t_0 and t_{28} . NM: not measured

	NC (mg/kg d.w.)				
	1.85	5.56	16.70	25.30	50.00
MC t_0	2.10	6.24	17.53	28.50	46.03
MC t_{28}	NM	6.05	NM	NM	45.80

Loss On Ignition of sediments was $4.9 \pm 0.2\%$ d.w. and remained constant until the end of the exposures. In all test beakers, the pH values of overlying water were 7.8 ± 0.2 on the first day of exposure and 8.0 ± 0.1 (*Tt* test), 7.9 ± 0.2 (*Lv* test) and 8.0 ± 0.2 (*Crip* test) at the end of the tests.

Oxygen concentration always exceeded 7.1 mg/L and ammonium was detected (0.06 mg/L) only in the *Tt* test at the highest PCP concentration, most likely because of the mortality of all test organisms. Otherwise, the observed exposure conditions exclude adverse effects to the organisms due to anoxia, to low availability of organic matter or pH fluctuations. The solvent caused no adverse effects to any of the test organisms (Tables S2–S5 – Supplementary Material).

In the *Tt* bioassay, all breeding adults died at the maximum exposure concentration (46.03 mg/kg d.w.) and neither cocoons nor young worms could be observed. A reduced number of cocoons and young worms was recorded with increasing concentrations of PCP in sediments for the other exposure levels (Figure 1). The same situation was observed in the *Lv* test, where increasing concentrations resulted in a decreasing number of worms and average dry weight (Figure 1). The

main difference between the two bioassays was that individuals of *L. variegatus* were still present at the highest exposure concentration, albeit in a reduced number. In both tests, the reworking activity of adult worms was reduced as visually observed by the decreased number of galleries in sediments. In the *Crip* tests, a decrease in growth (10 days) and a delay in emergence (28 days) were also observed with increasing exposure concentration (Figure 1), with emergence being completely suppressed at the maximum exposure concentration (46.03 mg/kg d.w.). On the other hand, no significant differences existed between exposure concentrations for the survival of the midges in the 10 day test (Table S5).

Figure 1. Response (relative to the corresponding controls) of the various end-points for the different organisms exposed to PCP (average values, $n=5$; refer to Tables S2–S6 for individual data points). All concentrations are in mg/kg d.w.; note the logarithmic scale on the x-axis which necessitates an axis break to properly show control data. *Tt*, *T. tubifex*; *Lv*, *L. variegatus*; *Crip*, *C. riparius*.

3.2. Data derivation for PCP risk assessment

Risk assessment refers to the determination of the ecotoxicological effects of a substance on biota, including the analysis of adverse effects at different exposure concentrations and for different species and endpoints (Woin, 1998). We obtained different EC₅₀ and EC₁₀ estimates, foundational to REACH regulation, from multiple dose–response experiments which exhibited a quite clear and complete concentration–response relationships (Figure 1). These data allow to estimate the potential impact of PCP on benthic organisms.

Table 2 Estimated EC₅₀ (mg PCP kg⁻¹ d.w.) and EC₁₀ (mg PCP kg⁻¹ d.w.) with their respective lower and upper 95% confidence intervals (in parentheses) for the various endpoints and model organisms.

Organism	End point	EC ₅₀	EC ₁₀
		(Lower and upper 95% ci)	(Lower and upper 95% ci)
<i>Tubifex tubifex</i>	Number of cocoon	27.5 (22.9-33.1)	16.7 (11.6-24.1)
	Number of young worms	2.9 (2.06-4.03)	0.3 (0.2-0.6)
<i>Lumbriculus variegatus</i>	Number of total organisms	26.4 (20.1-34.7)	10.1 (7.3-14.1)
	Wet weight	23.3 (20.1-26.9)	11.0 (8.9-13.7)
<i>Chironomus riparius</i>	Wet weight (10 days exposure)	19.1 (9.4-38.9)	2.6 (1.0-6.5)
	Emergence (28 days exposure)	4.4 (3.5-5.3)	2.1 (1.0-3.1)

The different end-points showed a different sensitivity for *T. tubifex* and *C. riparius*, but not for *L. variegatus*. Among oligochaetes, the most sensitive end-point was the number of young *T. tubifex* worms, while no significant differences existed between the other end-points (EC₅₀ range 23–27.5 mg PCP/kg d.w.). The emergence of *C. riparius* gave a value similar to the number of young *T. tubifex* worms, while the growth of chironomids' larvae after a 10-day exposure was not statistically different from the end-points measured for oligochaetes. Results for both endpoints in *L. variegatus* were in excellent agreement with those of a ring-test involving 14 laboratories and yielding a 28-d EC₅₀ (arithmetic means $\pm$ 1 standard deviation) of 23 $\pm$ 10.7 mg/kg d.w. and 20.4 $\pm$ 9.1 mg/kg d.w. for total number of worms and total dry weight of worms, respectively (Egeler et al., 2005). According

to these results, *L. variegatus* therefore appears less sensitive to PCP than the other benthic organisms examined in this study.

On the other hand, Mäenpää et al. (2008) reported full mortality for *L. variegatus* starting from 25 mg/kg d.w. and calculated a 28-d EC₅₀ for growth of 1.7 mg/kg d.w. Nikkilä et al. (2003) reported a 96h LC₅₀ of 8.1 mg/kg d.w. for *L. variegatus* exposed to a natural sediment spiked with PCP. The highest sensitivity observed in these previously published studies likely may originate from several factors such as differences in equilibration time after spiking, sediment characteristics and overlying water pH. Contrary to the present study, both Nikkila et al. (2003) and Mäenpää et al. (2008) observed substantial PCP losses (40 to 90% of the initially added concentrations) from sediments at the end of the test period. Part of the observed ecotoxicity could therefore have originated from an exposure via the aqueous route, in which PCP ecotoxicity is pH dependent (Xing et al., 2012). Benthic organisms are always exposed to contaminants via multiple routes and our considerations are meant to facilitate possible comparisons of ecotoxicity data with *L. variegatus*. Apparent reduction in the emergence rate of *C. prasinus* exposed to artificial sediment was reported for PCP levels between 5 and 20 mg/kg. A decrease in the number of egg ropes was also observed in the concentration range 1.25–20 mg/kg d.w. However, no clear concentration-response relationships could be observed for either endpoints (Sánchez et al., 2005). Considering that *C. prasinus* and *C. riparius* are closely related (they belong to the same genus), the observed interspecific differences probably arise from the different exposure conditions, as already observed for *L. variegatus*.

Analysing the no effect concentrations in terms of EC₁₀, the most stringent value is given by the number of young worms of *T. tubifex* (significantly different from the other end-points), followed by the emergence and wet weight of *C. riparius* (Table 2). Table 3 shows the ratio of EC₁₀ estimates between young *T. tubifex* worms and the other end-points along with the corresponding 95% confidence intervals. An upper 95% confidence limit smaller than 1 indicates that the EC₁₀ for the number of young *T. tubifex* worms is significantly lower than those for the number of *T. tubifex* cocoons and the two end-points for *L. variegatus* ($p < 0.05$). On the other hand, no statistically

significant differences are observed between the EC₁₀ for young *T. tubifex* worms and the *C. riparius* end-points.

Table 3 Estimated ratios (with upper and lower 95% confidence intervals–CI) between the EC₁₀ value of young *T. tubifex* worms and the other end-points for the different model benthic organisms. EC₁₀ ratios have been calculated from the corresponding values reported in Table 2. The lower and upper confidence intervals (CI) have been estimated using the R package *drc* (see section 2.5).

Organism	End point	EC ₁₀ Ratio	Lower 95%CI	Upper 95%CI
<i>Tubifex tubifex</i>	Number of cocoon	0.018	0.002	0.210
<i>Lumbriculus variegatus</i>	Number of total organisms	0.030	0.002	0.411
	Wet weight	0.027	0.002	0.345
<i>Chironomus riparius</i>	Wet weight (10 days exposure)	0.118	0.010	1.450
	Emergence (28 days exposure)	0.243	0.023	2.521

3.3 Proposal for sediment-based Environmental Quality Standard for PCP

The derivation process of sediment Quality Standards (QS) is detailed in the Technical Guidance for Deriving Environmental Quality Standards prepared by the Common Implementation Strategy for the Water Framework Directive (European Commission, 2011).

Since three long-term tests have been performed with species representing different living and feeding conditions, a QS for PCP can be obtained dividing the lowest EC₁₀ of 0.30 mg/kg d.w. (*Tt* young end-point) by an assessment factor of 10 (European Commission, 2011). The resulting QS of 0.030 mg/kg d.w. (30 ng/g d.w.) is about one fourth of the tentative QS of 119 ng/g d.w. calculated by applying the equilibrium partitioning approach to data for organisms living in the water column (European Commission, 2005).

However, no standard OECD guideline exists for *T. tubifex*. Considering the lowest EC₁₀ value of 2.06 mg/kg obtained for the OECD standard test organism *C. riparius*, the QS would be 0.0412 mg/kg (41.2 ng/g d.w.), obtained by applying an Assessment Factor of 50 (two species, *C. riparius*

and *L. variegatus* - representing different living and feeding conditions). This concentration is not far from the one defined using the endobenthic *T. tubifex* and from the PNEC of 0.025 mg/kg proposed by Muir and Eduljee (1999) for freshwater sediments using an equilibrium partitioning approach based on a PNEC water of 1 µg/L, a K_{oc} of 500 L/kg and a sediment total organic carbon content of 5%. It is also noteworthy that the organic matter content of the sediment used in this study (approx. 5 %) is about half of the 10% value assumed by the EU PCP data sheet for applying the Equilibrium Partitioning approach (European Commission, 2005). Without additional confounding factors, estimation of a QS for sediments with an organic matter content of 5 %, following the EU procedure (see Supporting Material), would yield a value of 60 µg/kg d.w., in reasonable agreement with the QS estimated from our experimental data. Starting from the results obtained in this study and applying the equilibrium partitioning approach according to the PCP substance data sheet (European Commission, 2005), the water based EQS for PCP would be about 0.1 µg/L (see calculations in the Supplementary Material). Otherwise stated, the current water-based EQS of 0.4 µg/L (EU Directive 2013/39) may be under-protective of benthic organisms such as *C. riparius*, *L. variegatus* and *T. tubifex*. Similar issues may apply when deriving site-specific EQS. Using the US EPA methodology, Chen et al. (2016) estimated a Continuous Concentration Criterion (CCC) of 2.07 µg/L (at pH 8.0) for PCP concentrations in the waters of Lake Tai (China). This value is lower than the current Chinese water quality criteria of 9 µg/L because it specifically accounted for effects on sensitive species that occurred in Lake Tai in the 1980s. Our results suggest that such site-specific criteria would also benefit from additional verification using sediment-based tests with benthic invertebrates. We acknowledge however that sediment-based EQS derived using standard sediments also need to be adjusted to the characteristics of the local natural sediments.

4. Conclusions

Current European regulation establishes environmental quality standards (EQS) for PCP in terms of total concentrations in unfiltered water samples, assuming that such EQS will be equally protective

for benthic organisms. Based on the current EQS value of 0.4 µg/L, an indicative quality standard of 119 µg PCP/kg d.w. has been calculated for the sedimentary compartment using the equilibrium partitioning approach. The experimental data generated by this study converge around QS values of 30–40 µg PCP/kg d.w. and suggest that additional testing with benthic organisms is required to verify the assumption that water-based EQS also protect sediment-dwelling organisms, even considering the recently analysed concentrations in sediments of some sites (Table S1). Furthermore, application of the EqP approach requires an educated guess of the sediment-water PCP partition coefficient to be used in the calculations. Partition coefficients are, by definition, conditional with those for PCP being influenced by pH (PCP sorption to organic matter increases with decreasing pH) and by the organic matter content of the sediments. These considerations also support the recommendation to test more benthic organisms (and more sediment types) for their sensitivity to PCP, particularly in consideration of the long-term environmental persistence of this contaminant in the sedimentary compartment. A final word of caution concerns the use of the OECD standard species *L. variegatus* which appears to be less sensitive than other organisms to PCP and could be less appropriate to generate information on the long-term ecotoxicological effects of PCP.

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Supplementary material for “The European water-based environmental quality standard for pentachlorophenol is not protective for benthic organisms”

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Table S1. PCP levels in sediments samples after the year 1998. All values are in ng/g dry weight. Empty cells, information not available.

System	Country	Site name	Site type	Range	# samples per site	Mean $\pm$ s.d.	Year of sampling	References
Pear River Delta	China	Shunde	fishpond	1.55-3.62	5	2.2 $\pm$ 0.82	2000-2001	Hong et al., 2005
Pear River Delta	China	Shunde	river sediment	2.81-34.4	2	n.a.		
Pear River Delta	China	Zhongshan	fishpond	0.56-163	13	37.5 $\pm$ 52.6		
Pear River Delta	China	Zhongshan	river sediment	1.44-11.72	7	5.45 $\pm$ 3.42		
Pear River Delta	China	Donguan	fishpond	6.6-44.4	4	21.1 $\pm$ 15.7		
Pear River Delta	China	Donguan	river sediment	5.65-8.79	5	7.14 $\pm$ 1.25		
Pear River Delta	China	Shenzhen	fishpond	2.31-6.83	4	3.69 $\pm$ 2.11		
Puck Bay	Poland		coastal sediment			17.4 $\pm$ 2.6	2008	Maciak et al., 2016
Puck Bay	Poland		coastal sediment			230 $\pm$ 21		
Vistula River	Poland	Cracow	river sediment			1.5-1.6	2005	Dmitruk et al., 2008
Vistula River	Poland	Annopol	river sediment			1.2-1.8		
Vistula River	Poland	Warsaw	river sediment			$\leq$ 1.2		
Vistula River	Poland	Wloclawek	river sediment			0.6-2.2		
Vistula River	Poland	Gdansk	river sediment			0.9-1.3		
Lake Mayrut	Egypt	19 sites; unnamed	brackish lake	0.654*-200	19	50*	2009	Khairy, 2013
Yangtze river/Wuhan	China	11	river sediment	<0.64-1.68		0.38 $\pm$ 0.58	2005	Tang et al., 2007
Hanjiang river	China	4	river sediment	<0.64-0.87		0.34 $\pm$ 0.42		
Fuhe river	China	3	river sediment	<0.64-0.77		0.46 $\pm$ 0.40		
Sheshui river	China	1	river sediment	0.59				
Dongjing river	China	2	river sediment	<0.64-0.628				
Tsingshan port	China	1	river sediment	3.47				
Lakes	China	8	lake sediment	<0.64-12.78		3.47 $\pm$ 4.61		
Dongtin Lake	China	8 sites; unnamed	lake sediment	180-48,300	1	1 measure per site	1998	Zheng et al., 2000

*PCP-Na treated for eradication of schistosomiasis

- 1 Table S2. Data sheet of *Tubifex tubifex* test results of survival and reproduction. Repl: number of
 2 replicate; CC: number of cocoon; YW: number of young worms.

	Repl	CC	YW
Control	1	37	170
	2	36	173
	3	38	171
	4	35	175
	5	38	178
Solvent control	1	35	170
	2	34	165
	3	38	176
	4	37	175
	5	36	168
2.10 PCP (mg/kg d.w.)	1	34	89
	2	32	79
	3	35	90
6.24 PCP (mg/kg d.w.)	1	30	54
	2	29	68
	3	28	65
17.53 PCP (mg/kg d.w.)	1	24	32
	2	21	23
	3	24	22
28.50 PCP (mg/kg d.w.)	1	15	10
	2	14	10
	3	16	9
46.03 PCP (mg/kg d.w.)	1	0	0
	2	0	0
	3	0	0

4 Table S3. Data sheet of *Lumbriculus variegatus* test results for survival at 28 days. Repl: replicate
5 number.

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	Repl	Total n.
Control	1	37
	2	38
	3	35
Solvent control	1	38
	2	38
	3	36
2.10 PCP (mg/kg d.w.)	1	34
	2	35
	3	34
6.24 PCP (mg/kg d.w.)	1	34
	2	33
	3	32
17.53 PCP (mg/kg d.w.)	1	25
	2	26
	3	24
28.50 PCP (mg/kg d.w.)	1	18
	2	15
	3	22
46.03 PCP (mg/kg d.w.)	1	5
	2	6
	3	7

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18 Table S4. Data sheet of *L. variegatus* test results for growth at 28 days. Repl: replicate number.

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	Repl	Weight (mg d.w.)
Control	1	30.86
	2	26.60
	3	31.19
Solvent control	1	30.01
	2	29.54
	3	28.08
2.10 PCP (mg/kg d.w.)	1	28.52
	2	28.01
	3	27.81
6.24 PCP (mg/kg d.w.)	1	27.00
	2	27.06
	3	26.88
17.53 PCP (mg/kg d.w.)	1	19.95
	2	21.06
	3	18.72
28.50 PCP (mg/kg d.w.)	1	10.26
	2	9.00
	3	12.76
46.03 PCP (mg/kg d.w.)	1	2.20
	2	2.82
	3	2.80

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21 Table S5. Data sheet of *Chironomus riparius* test results for survival and growth after 10 days
 22 exposure. Repl: replicate number.

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	Repl	n. larvae	Wet weight w.w. (mg) per larva									
Control	1	10	3.0	2.5	1.2	3.0	2.5	2.5	1.9	3.4	3.3	2.6
	2	10	3.0	2.5	2.8	2.3	3.6	3.2	3.3	3.3	2.7	2.9
	3	9	2.1	3.1	2.9	3.1	4.2	2.6	3.2	3.8	3.5	
Solvent control	1	7	3.8	4.6	3.6	2.9	3.5	1.2	3.5			
	2	10	1.3	3.5	3.6	3.7	3.4	2.7	2.6	1.3	3.2	3.1
	3	9	1.3	0.9	1.0	4.2	4.0	3	2.7	3.6	3.8	
2.10 PCP (mg/kg d.w.)	1	10	1.0	2.8	1.8	1.3	2.3	2.3	2.4	2.2	2.0	1.9
	2	8	0.6	3.2	2.9	4.2	3.5	3.0	3.2	3.9		
	3	9	1.3	4.1	2.3	2.5	3.1	1.8	1.7	2.4	2.3	
6.24 PCP (mg/kg d.w.)	1	9	2.2	2.6	2.3	2.3	2.6	2.2	1.8	2.3	1.9	
	2	8	2.4	1.7	3.3	2.0	2.5	0.6	1.5	1.5		
	3	10	2.9	3.2	3.7	2.1	1.0	1.1	0.9	1.2	2.5	2.3
17.53 PCP (mg/kg d.w.)	1	10	0.7	1.5	2.4	3.2	2.3	1.9	2.1	1.8	0.9	1.0
	2	8	0.5	2.3	2.0	1.5	2.4	2.2	1.8	2.1		
	3	9	1.1	0.9	1.2	1.8	3.1	2.3	1.7	1.8	1.6	
28.50 PCP (mg/kg d.w.)	1	7	1.3	1.6	0.6	1.2	2.1	1.3	1.0			
	2	9	1.0	1.1	1.0	1.3	0.9	1.4	2.1	1.3	0.8	
	3	10	0.4	0.7	1.2	1.8	1.5	0.8	0.4	0.7	0.9	1.3
46.03 PCP (mg/kg d.w.)	1	9	0.3	0.4	0.3	0.2	0.9	0.4	1.0	0.2	0.3	
	2	10	0.7	0.5	1.1	1.0	0.8	0.4	0.3	0.2	0.2	0.2
	3	10	0.5	0.9	1.2	0.9	0.6	0.2	0.7	0.9	0.3	0.4

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35 Table S6. Data sheet of *Chironomus riparius* test results for emergence after 28 days exposure.

36 Repl: number of replicate

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	Repl	n. emerged larvae
Control	1	10
	2	9
	3	9
	4	9
	5	10
Solvent control	1	9
	2	9
	3	10
	4	9
	5	10
2.10 PCP (mg/kg d.w.)	1	9
	2	8
	3	8
	4	10
	5	9
6.24 PCP (mg/kg d.w.)	1	5
	2	3
	3	3
	4	3
	5	2
17.53 PCP (mg/kg d.w.)	1	3
	2	2
	3	4
	4	2
	5	2
28.50 PCP (mg/kg d.w.)	1	1
	2	2
	3	2
	4	1
	5	1
46.03 PCP (mg/kg d.w.)	1	0
	2	0
	3	0
	4	0
	5	0

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Application of the Equilibrium Partitioning Approach to estimate a tentative water-based EQS starting from the ecotoxicological results obtained with benthic organisms

According to the substance data sheet for pentachlorophenol (PCP) compiled by the common implementation strategy for the Water Framework Directive (European Commission, 2005), the relationship between water-based and sediment-based environmental quality standard (QS) is described by the formula:

$$QS_{sed.wet.weight} \left[\frac{mg}{kg} \right] = \frac{K_{SPM-water}}{bulk\ density_{SPM.wet}} * 1000 * QS_{water} \left[\frac{mg}{L} \right] \quad [1]$$

With:

$K_{SPM-water} = f_{solid} (0.1) * K_{p_{susp}} (340\ L/kg) / 1000 * RHO_{solid} (2500\ kg/m^3) = 85\ m^3/m^3$ (see European Commission, 2005 for details)

$Bulk\ density_{SPM.wet} = 1150\ kg/m^3$

$1000 = \text{conversion factor } m^3/kg \text{ to } L/kg$

$QS_{water} = 0.00035\ mg/L$

Substituting the numerical values into equation one yields a $QS_{sed.wet.weight}$ of $25.9\ \mu g/kg\ w.w.$

European Commission (2005) also assumes a wet:dry ratio of 4.6; thus giving a $QS_{dry.weight}$ of $119\ \mu g/kg\ d.w.$

The tentative $QS_{sed.dry.weight}$ obtained in this study are (see section 3.3 of the main text):

$0.030\ mg/kg\ d.w$ for the number of juveniles in the test with *Tubifex tubifex*

$0.041\ mg/kg\ d.w.$ for the emergence at 28 days in the test with *Chironomus riparius*

These values correspond to:

$0.030 / 4.6 = 0.006\ mg/kg\ w.w.$ for *T. tubifex* and

$0.272 / 4.6 = 0.008\ mg/kg\ w.w.$ for *C. riparius*

Rearranging formula [1] to calculate QS_{water} from $QS_{sed.wet.weight}$ the corresponding QS for water would be:

$$QS_{water} \left[\frac{mg}{L} \right] = \frac{0.006}{1000} * \frac{1150}{85} = 0.00008\ \frac{mg\ PCP}{L} \text{ based on the } T. tubifex \text{ endpoint}$$

$$QS_{water} \left[\frac{mg}{L} \right] = \frac{0.008}{1000} * \frac{1150}{85} = 0.0001\ \frac{mg\ PCP}{L} \text{ based on the } C. riparius \text{ endpoint}$$

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