Effects of salinity, DOM and metals on the fate and microbial toxicology of propetamphos formulations in river and estuarine sediment

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ABSTRACT

Toxicity studies tend to use pure pesticides with single organisms. However, natural systems are complex and biological communities diverse. The organophosphate pesticide propetamphos (PPT) has been found exceeding regulatory limits (100 ng L^{-1}) in rivers. We address whether solution properties affect the fate of Analar (Analar-PPT) or industrial PPT (PPT-Ind) propetamphos formulations and whether propetamphos and metal toxicant effects are additive, antagonistic or synergistic? The sorption, desorption, biodegradation and microbial toxicology of Analar-PPT and PPT-Ind were investigated in Conwy River and estuary sediment. Results showed elevated salinity enhanced PPT sorption, while higher salinities increased PPT-Ind retention. Higher dissolved organic matter (DOM) and low salinity slowed Analar-PPT biodegradation (1.9×10^{-3} h^{-1}). Analar-PPT and PPT-Ind biodegradation was further reduced by low salinity, high DOM and dissolved Zn and Pb (6.3×10^{-4} h^{-1}, 1100 h $t_{1/2}$ for Analar-PPT; 7.5×10^{-4} h^{-1}, 924 h $t_{1/2}$ for PPT-Ind). Toxicity effects of PPT, Zn and Pb in equitoxic ratio were higher for PPT-Ind (4.7 µg PPT-Ind g^{-1}; 581 µg Zn g^{-1}; 395 µg Pb g^{-1}) than for Analar-PPT (34.6 µg PPT g^{-1}; 312 µg Zn g^{-1}; 212 µg Pb g^{-1}) whilst a toxicant ratio 1:100:10 suggested small quantities of Analar-PPT ($EC_{10} = 0.06$ µg g^{-1}) affected microbial communities. The combined toxicity effect was more than additive. Thus, industrial formulations and pollutant mixtures should be considered when assessing environmental toxicity.

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1. Introduction

Organophosphate (OPs) have largely replaced organochlorine pesticides as they have shorter environmental half lives (Fest and Schmidt, 1982). However, concern about OP ecotoxicity has led to their replacement with alternatives (e.g. synthetic pyrethroids). Nevertheless, OPs are still widely used in the UK and other countries, due to concern about the environmental impact of synthetic pyrethroids (Ventura-García et al., 2008). In the USA, propetamphos (PPT), is an indoor insecticide not implicated in water pollution (EPA, 2008). In the UK, PPT has been used as a sheep insecticide (Virtue and Clayton, 1997) and, in 1999, 9% of UK Environmental Agency monitored rivers in agricultural catchments failed PPT environmental limits (EA, 1999).

OP environmental toxicity and fate depends on factors such as solid–liquid partitioning, other contaminants (e.g. metals), temperature, water quality and microbial activity (Singh and Walker, 2006). Aquatic OP mobility is influenced by pH, ionic strength, redox and complexing agents (e.g. dissolved organic matter; DOM) (Salomons, 1995). Consequently, assessing OP transport, bioavailability and toxicology in freshwater and marine waters remains difficult producing uncertainty for OP environmental impact.

Typically, chemical toxicity for an organism is measured by acute, sub-chronic and chronic effects with exposure defined relative to the test organism's life cycle (Rand, 1995). However, in nature, many compounds can simultaneously affect an organism's toxic response where the combined effect of two or more pollutants can exceed (synergistic effect) or lessen (antagonistic effect) that when acting alone (Mason, 1996). Unfortunately, contaminant risk assessment rarely incorporates this complexity (den Besten et al., 2003).

Estuaries (e.g. the Conwy estuary) play an important role in contaminant transport (Noegrohati et al., 2008). Adsorption, flocculation, precipitation and biochemical processes aid pollutant removal with the estuary effectively acting as a filter (Sharp et al., 1984) whilst desorption makes estuarine ecosystems sensitive to pollution (Geffard et al., 2005). Here we address whether solution properties influence Analar- or industrial-grade PPT fate and whether the toxic effects of propetamphos and metals are additive, antagonistic or synergistic?

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2. Materials and methods

2.1. Field site and sampling

The Conwy estuary (North Wales, UK) drains a predominantly upland catchment (590 km^2) discharging into Liverpool Bay at Conwy (53°16′54″N, 3°49′41″W) after flowing 56 km (NRA, 1995). The upper limit of tidal influence is 10 km upstream from the main body of estuarine water and the maximum spring tidal range is 7–8 m (Elderfield et al., 1971). High-intensity sheep grazing, where sheep dip OP insecticides have been widely used, dominates the catchment with abundant disused mines (Zn and Pb) in sub-catchments; known sources of water pollution.

Sites along the Conwy river-estuary included a freshwater site 10 km above the upper tidal limit (pH 6.9; pEh 158 mV; salinity 0.6 g L^{-1}; 3.4 mg L^{-1} DOC), one in the high estuary within the tidal zone (pH 7.4; pEh 159 mV; salinity 10.1 g L^{-1}; 6.2 mg L^{-1} DOC) and a lower estuary site (pH 7.8; pEh 166; salinity 29.7 g L^{-1}; 4.0 mg L^{-1} DOC).

Sediment samples were collected as intact cores (10 cm diameter, 0–10 cm depth) with a high-density polyethylene corer. Replicate cores were bulked and transported (5 °C) to the laboratory. Samples were wet sieved (2 mm) and stored (5 °C).

2.2. Chemicals

Propetamphos, (1-methylethyl (e)-3{{(ethylamino)meth-oxyphosphinothioyl}oxy}-2-butenoate) or PPT, was used as Analar-PPT (Pestanal grade®, Sigma, UK; 95.9% purity) or its industrial formulation (PPT-Ind; Ectomort centenary®, 8%, Vericore, UK). ^{14}C-labelled PPT (Huntingdon Life Sciences, UK; specific radioactivity 0.732 MBq kg^{-1}; radiochemical purity 97.9%), was used to isotopically label PPT solutions. ^{14}C activity was measured with a Wallac 1409 liquid scintillation counter (Perkin-Elmer Life and Analytical Sciences, USA) with Wallac Optiphase 3 scintillation fluid (EG&G Ltd., UK).

2.3. PPT sorption and desorption

Experiments were run in Pyrex® bottles (25 mL) with Teflon® liners. Distilled water was used throughout. To measure sorption, sediment (2 g) was mixed with ^{14}C-labelled PPT (20 mL, 0.5 µg mL^{-1}) and shaken (150 rev min^{-1}) in the dark at 20 °C for ⩽24 h with NaN$_3$ (40 mM) to minimize microbial activity. Sediment solution (1.5 mL) was centrifuged at 10,000g for 5 min and supernatant (1 mL) mixed with scintillation cocktail. Non-sorbed ^{14}C-PPT was measured (three replicates) and sorbed PPT (expressed as sediment dry weight basis) calculated by difference.

Salinity effects on PPT sorption were measured in a background solution of 0, 10, 20, 30 and 40 g kg^{-1} artificial sea salts (Sigma–Aldrich, MO), in high and low estuary sediment after 5 min, 12 h or 24 h.

PPT desorption was measured after 48 h PPT exposure during the sorption experiments by centrifuging samples (9000g, 10 min), removing the PPT solution and removing 1.0 mL for ^{14}C measurement (t0). PPT solution was substituted with 20 mL of pesticide-free saline solution and the samples shaken (150 rev min^{-1}). After 24 h (t1), sediment solution (1.5 mL) was taken, centrifuged and desorbed Analar-PPT measured by ^{14}C activity; sorbed PPT was calculated by difference.

One aim was to study natural DOM effects on PPT. Standardized DOM was used which, although purified, had the properties of the natural source material. Thus, Analar-PPT (0.1 µg mL^{-1}) sorption in high estuary sediment was measured as described above in the presence humic acid (Na salt, Cat. No. H16752; Sigma–Aldrich) at

0, 5, 10, 30 and 50 mg C L^{-1}. Effects of DOM origin (40 mg C L^{-1}) were tested using humic acid (Sigma–Aldrich), fulvic acid and humic acid from Lake Skervatjern, Western Norway part of HUMEX (Humic lake, acidification experiment), and dissolved organic matter from Suwannee River (DOM-SR), South Georgia USA (obtained from the International Humic Substance Society) as described by Chi and Gary (2004).

To compare treatments, one way ANOVA tests with Tukey pairwise comparison were undertaken with MINITAB 13; $P < 0.05$ was the limit for statistical significance.

2.4. Matrix effects on PPT biodegradation

Biodegradation measured ^{14}C-labelled PPT mineralization to $^{14}CO_2$ (three replicates). Wet sediment from the freshwater site (2 g dry weight) was placed in 25 mL Pyrex bottles and spiked with 2 mL of 222 Bq ^{14}C-labelled PPT, 1.0 µg g^{-1} Analar-PPT. After hermetically sealing the bottles, $^{14}CO_2$ was captured in a 1 M NaOH trap (1 mL) above the sediment and measured by liquid scintillation counting as described by Rhodes et al. (2010).

Aqueous matrix effects on PPT biodegradation kinetics were tested on Analar-PPT (1.0 µg g^{-1}; 222 Bq) spiked sediment in four matrices; double distilled water (DDW), artificial sea water (ASW) at 3.3 g L^{-1} of artificial sea salt (ASS) (Sigma), DOM-SR (130 mg C L^{-1}) and ASW (3.3 g L^{-1}) with DOM-SR (130 mg C L^{-1}). Sediment (2 g) to aqueous (2 mL) was 1:1 (w:v) for all experiments. Microbial degradation ($^{14}CO_2$ evolution) was measured after 48, 120, 192 and 240 h.

Zinc (2.2 or 4.4 µg g^{-1}) or lead (5.5 or 11.1 µg g^{-1}) effects on Analar-PPT biodegradation were measured using wet river sediment (2 g dry weight) spiked with ^{14}C enriched Analar-PPT (222 Bq, 1.0 µg g^{-1}). $^{14}CO_2$ evolution was measured after 120 and 240 h.

Metal (2.2 µg Zn g^{-1} or 5.5 µg Pb g^{-1}), DOM-SR (130 mg C L^{-1}) or salinity (ASW 3.3 g L^{-1}) effects on ^{14}C-labelled (222 Bq) Analar-PPT and PPT-Ind (1 µg g^{-1}) biodegradation were measured over 10 d with $^{14}CO_2$ sub-samplings after 48, 120, 192 and 240 h. Control sediment was spiked with uniformly labelled ^{14}C-glucose (0.25 mM; 196 kBq; specific activity 11 × 10^9 Bq mmol^{-1}; Sigma–Aldrich) to measure total microbial activity for a readily mineralized organic substrate.

Biodegradation data were linearized by least-square regression. Normality and homogeneity of variances were tested. Analysis of covariance (ANCOVA) was used to compare biodegradation rates. Regressions described biodegradation rates in specific matrices with time being covariate. Differences between treatments, within time periods, were determined after the Wilcox procedure (Wilcox, 1987).

2.5. Toxicological effects of binary and ternary combinations of PPT and selected metals

Analar-PPT, PPT-Ind, Zn and/or Pb toxicological effects on indigenous sediment microbes were measured individually, in binary and in ternary combinations in DDW and with DOM-SR at 1:1 (w/v) sediment:water ratio. ^{14}C-glucose mineralization measured microbial activity (1 h incubation, four replicates). Respiration ($^{14}CO_2$ production) was measured by NaOH capture (1 mL, 1 M) and compared with control (no PPT) (Prakash et al., 1996).

To establish EC$_{50}$ for binary and ternary experiments, PPT formulations and metals were tested individually. DDW Concentrations were; Analar-PPT (0, 0.1, 1, 10, 60, 110 and 140 µg g^{-1}), PPT-Ind (0.1, 1, 10, 60, 110, 140, 500 and 1000 µg g^{-1}), Zn and Pb (10, 50, 100, 500, 1000 and 2000 µg g^{-1}). DOM-SR (40 mg C L^{-1}) effects on Analar-PPT, Pb or Zn toxicity were tested as described above.

Percentage $^{14}CO_2$ production inhibition from ^{14}C-glucose at different toxicant concentrations was calculated relative to the control (sediment + water) and plotted *versus* toxicant concentration (Log_{10}; mg g^{-1}). Concentrations inhibiting 50% or 10% of $^{14}CO_2$ production determined EC_{50} or EC_{10}.

Marking and Dawson's (1975) model was used to evaluate joint toxicant action. It is based upon the toxic unit concept. The sum of action of components of a mixture is represented by

$$\frac{Am}{Ai} + \frac{Bm}{Bi} = S \tag{1}$$

where A and B are chemicals, and i and m are their respective toxicities (EC_{50}) individually (i) or in a mixture (m), and S is the sum of activity. Therefore, if A and B are equitoxic with similar modes of toxic action, their fractional combination would have the same effect as one toxic unit of either. These values can be substituted into formula (1): e.g. if each chemical contributes ½ toxic units,

$$\frac{Am}{Ai} + \frac{Bm}{Bi} = \frac{1}{2} + \frac{1}{2} = 1.0 \text{ of } S \tag{2}$$

The toxicological effect of binary combinations of Analar-PPT and metals in DDW were studied at 1:1 equitoxic ratios calculated from their EC_{50} values, and testing a range of toxic units TU (0.25, 0.5, 0.75, 1.0, 1.25, 1.5 and 1.75). The TU of a mixture was calculated by adding the ratios of the concentrations of each metal in the mixture divided by its EC_{50}. Ternary combinations were carried out at 1:1:1 equitoxic ratio in DDW and with DOM-SR (40 mg C L^{-1}) with Analar-PPT or PPT-Ind and at 1:100:10 ratio for Analar-PPT:Zn:Pb, mimicking their environmental ratio.

Marking and Dawson (1975) state a positive additive index (AI) means the toxic effect of the mixture is greater than additive; negative AI means the effect is less than additive, and if, AI = zero, the effect is additive.

3. Results and discussion

3.1. Sorption and desorption

Table 1 shows Analar-PPT sorption on high estuary sediment was increased by salinity ($\geqslant 30$ g ASS L^{-1}) mostly after 24 h ($P < 0.05$). For low estuary sediment, salinity effects on Analar-PPT sorption were only noticeable at 24 h when the salinity was 20 g ASS L^{-1} with little effect at higher salinities ($P > 0.05$). Salinity had little effect on Analar-PPT desorption ($P > 0.05$) in both sediments although high estuary sediment suggested more Analar-PPT remained sorbed at t_0 with 30 and 40 g ASS L^{-1} ($P < 0.05$). For desorption, sorbed Analar-PPT at t_0 was always greater than t_1 because samples had another 24 h to equilibrate.

Table 2 shows salinity effects on PPT-Ind sorption (0.5 µg mL^{-1}) decreased with time with significant PPT-Ind sorption at 40 g ASS L^{-1} at 24 h ($P < 0.05$). After 24 h, PPT-Ind at 0 g L^{-1} ASS and 40 g L^{-1} was more sorbed to high estuary sediment (1.24 ± 0.06 µg g^{-1}, 1.59 ± 0.15 µg g^{-1}) (Table 2) than Analar-PPT at the same salinities (0.90 ± 0.21 µg g^{-1}, 1.31 ± 0.11 µg g^{-1}) (Table 1) presumably reflecting the PPT-Ind formulation; important because few studies tackle this. At 40 g ASS L^{-1}, slight hysteresis may have been observed with sorbed PPT-Ind higher at 48 h (0.76 µg g^{-1}) than 24 h (0.70 µg g^{-1}) suggesting high salinities aid PPT-Ind retention.

ANOVA results (not shown) testing DOM effects on Analar-PPT sorption and desorption in high estuary sediment suggested no significant differences between treatments ($P > 0.05$); surprising as

Table 1
Effect of salinity on sorption and desorption of Analar-PPT (initial concentration 0.5 µg mL^{-1}, 5 µg g^{-1}) in high or low estuary sediment.

	Time	Artificial sea salt concentration (g L^{-1})				
		0	10	20	30	40
High estuary sediment (µg g^{-1})						
Sorbed PPT	12 h	0.76 ± 0.07[a]	1.19 ± 0.11[ab]	1.23 ± 0.22[b]	1.24 ± 0.08[b]	1.45 ± 0.27[b]
Sorbed PPT	24 h	0.90 ± 0.21[a]	1.20 ± 0.07[ab]	1.25 ± 0.12[ab]	1.41 ± 0.18[b]	1.31 ± 0.11[b]
PPT left sorbed	t_0, 0 h desorption	1.09 ± 0.10[a]	1.42 ± 0.08[ab]	1.33 ± 0.08[ab]	1.47 ± 0.23[b]	1.58 ± 0.08[b]
PPT left sorbed	t_1, 24 h desorption	0.32 ± 0.01[a]	0.57 ± 0.11[a]	0.42 ± 0.10[a]	0.46 ± 0.20[a]	0.49 ± 0.13[a]
Low estuary sediment (µg g^{-1})						
Sorbed PPT	12 h	1.10 ± 0.06[a]	1.10 ± 0.22[a]	1.18 ± 0.20[a]	0.80 ± 0.16[a]	1.15 ± 0.17[a]
Sorbed PPT	24 h	0.94 ± 0.05[a]	1.12 ± 0.09[ab]	1.39 ± 0.17[b]	1.19 ± 0.09[ab]	1.24 ± 0.17[ab]
PPT left sorbed	t_0, 0 h desorption	1.23 ± 0.01[a]	1.28 ± 0.12[a]	1.28 ± 0.10[a]	1.33 ± 0.08[a]	1.32 ± 0.11[a]
PPT left sorbed	t_1, 24 h desorption	0.32 ± 0.03[a]	0.29 ± 0.11[a]	0.30 ± 0.09[a]	0.33 ± 0.11[a]	0.32 ± 0.11[a]

Values represent means ± standard deviation ($n = 3$). Values in a row followed by different letters indicate significant differences between treatments ($P < 0.05$).

Table 2
Effect of salinity on sorption and desorption of PPT-Ind (initial concentration of 0.5 µg mL^{-1}, 5 µg g^{-1}) in high estuary sediment.

	Artificial sea salt concentration (g L^{-1})				
	0	10	20	30	40
Sorbed PPT-Ind (µg g^{-1})					
5 min	0.86 ± 0.09[ab]	0.56 ± 0.10[a]	0.95 ± 0.21[b]	0.94 ± 0.15[b]	1.01 ± 0.10[b]
2 h	0.82 ± 0.15[ab]	0.59 ± 0.28[a]	0.96 ± 0.11[ab]	1.05 ± 0.08[ab]	1.22 ± 0.03[ab]
4 h	0.88 ± 0.03[a]	0.66 ± 0.07[a]	1.09 ± 0.12[ab]	1.09 ± 0.15[ab]	1.25 ± 0.04[b]
24 h	1.24 ± 0.06[a]	1.12 ± 0.16[a]	1.30 ± 0.13[a]	1.49 ± 0.14[a]	1.59 ± 0.15[b]
Desorption – PPT left sorbed (µg g^{-1})					
5 min	1.19 ± 0.05[ab]	1.04 ± 0.16[a]	1.23 ± 0.14[ab]	1.42 ± 0.14[ab]	1.53 ± 0.15[ab]
1 h	0.51 ± 0.03[ab]	0.32 ± 0.14[a]	0.47 ± 0.14[ab]	0.65 ± 0.03[ab]	0.80 ± 0.21[ab]
24 h	0.47 ± 0.02[a]	0.28 ± 0.18[a]	0.43 ± 0.20[a]	0.66 ± 0.15[a]	0.70 ± 0.16[a]
48 h	0.47 ± 0.11[a]	0.29 ± 0.06[a]	0.43 ± 0.17[a]	0.62 ± 0.06[ab]	0.76 ± 0.13[b]

Values represent means ± standard deviation ($n = 3$). Values in a row followed by different letters indicate significant differences between treatments ($P < 0.05$).

Table 3
Effect of DOM (50 mg C L^{-1}) on Analar-PPT (initial concentration 0.1 µg mL^{-1}, 1 µg g^{-1}) sorption and desorption in river sediment.

	Control (DDW)	Humic acid (Aldrich)	Humic acid (Norway)	Fulvic acid (Norway)	DOM (Suwannee)
Sorption (µg g^{-1})					
24 h	0.72 ± 0.03[a]	0.68 ± 0.04[a]	0.69 ± 0.04[a]	0.70 ± 0.02[a]	0.67 ± 0.02[a]
Desorption – PPT left sorbed (µg g^{-1})					
5 min	0.77 ± 0.03[a]	0.73 ± 0.03[a]	0.73 ± 0.03[a]	0.82 ± 0.05[a]	0.72 ± 0.01[a]
1 h	0.53 ± 0.02[ab]	0.48 ± 0.03[a]	0.50 ± 0.03[a]	0.59 ± 0.06[b]	0.49 ± 0.01[a]
24 h	0.45 ± 0.01[ab]	0.40 ± 0.03[a]	0.42 ± 0.05[ab]	0.51 ± 0.06[b]	0.46 ± 0.01[ab]

Values represent means ± standard deviation (n = 3). Values in a row followed by different letters indicate significant differences between treatments ($P < 0.05$).

Table 4
Linear equations for biodegradation of glucose (0.25 mM) and Analar-PPT (1.0 µg g^{-1}); and Analar-PPT (1.0 µg g^{-1}) and PPT-Ind (1.0 µg g^{-1}) with metals (2.2 µg Zn g^{-1} and 5.5 µg Pb g^{-1}) in river sediment from the freshwater site. C_o is initial analyte concentration, C is final concentration, t is time (hours).

	Linear equation	r^2	S	Half-life (h)
Analar-PPT and glucose biodegradation				
Analar-PPT				
DDW	ln C/C_o = 0.152–4.43 × 10^{-3} t	0.93	0.087	156
ASW	ln C/C_o = 0.054–2.36 × 10^{-3} t	0.92	0.057	294
DOM-SR	ln C/C_o = 0.087–2.87 × 10^{-3} t	0.97	0.036	241
DOM-SR and ASW	ln C/C_o = 0.049–1.93 × 10^{-3} t	0.98	0.023	359
Glucose				
DDW	ln C/C_o = −0.784–3.61 × 10^{-3} t	0.89	0.108	192
ASW	ln C/C_o = −0.718–2.86 × 10^{-3} t	0.93	0.067	242
DOM-SR	ln C/C_o = −0.863–6.65 × 10^{-3} t	0.99	0.042	104
DOM-SR and ASW	ln C/C_o = −0.578–8.00 × 10^{-3} t	0.97	0.124	87
PPT biodegradation in presence of Zn and Pb				
Analar-PPT				
DDW	ln C/C_o = 0.015–0.94 × 10^{-3} t	0.99	0.007	737
ASW	ln C/C_o = 0.003–0.65 × 10^{-3} t	0.98	0.006	1066
DOM-SR	ln C/C_o = 0.014–0.94 × 10^{-3} t	0.99	0.003	737
DOM-SR and ASW	ln C/C_o = 0.003–0.63 × 10^{-3} t	0.96	0.010	1100
PPT-Ind				
DDW	ln C/C_o = 0.005–0.89 × 10^{-3} t	0.99	0.005	779
ASW	ln C/C_o = 0.002–0.76 × 10^{-3} t	0.97	0.011	912
DOM-SR	ln C/C_o = −0.002–0.88 × 10^{-3} t	0.99	0.006	788
DOM-SR and ASW	ln C/C_o = −0.001–0.75 × 10^{-3} t	0.99	0.006	924

DDW is double distilled water, ASW artificial sea water (3.3 g kg^{-1} artificial sea salt), DOM-SR (130 mg C L^{-1}). S = standard deviation of the line and r^2 = correlation coefficient. Time points = 48, 120, 192 and 240 h.

we expected humic material to influence sorption. A new experiment used three other DOM types but ANOVA results confirmed no humic material had a significant effect on Analar-PPT sorption ($P > 0.05$) suggesting PPT-humic interactions could be slower than expected (Table 3). However, Norwegian fulvic acid did affect Analar-PPT desorption, retaining higher sediment concentrations after 1 h ($P < 0.05$) whilst, after 24 h, there was no effect. Benson and Long (1991) reported humic-pesticide interactions can alter toxicity. Schlautman and Morgan (1993) reported that DOM affects the sorption of hydrophobic pollutants. However, those studies were carried out in water with no sediment or soil. Our findings suggest that solution DOM-PPT binding forces are weak whilst sediment organic matter increases sorption. Comparison of our data with other studies is impossible because no published literature comparing Analar and Ind-PPT exists.

3.2. PPT biodegradation

Glucose was selected as a reference as it provides an ubiquitous substrate for microbial metabolism, has negligible sorption or interactions with humic acid or metals (Meredith and Radosevich, 1998).

Table 4 shows that varying the aqueous matrix significantly affected PPT degradation kinetics ($P < 0.05$ ANCOVA). Both high DOM_SR (130 mg C L^{-1}) and low salinity (ASW = 3.3 g kg^{-1} artificial sea salt), slowed PPT biodegradation (2.8×10^{-3} h^{-1} and

2.3×10^{-3} h^{-1}, respectively), also when applied together DOM-SR and ASW (1.9×10^{-3} h^{-1}). The Analar-PPT $t_{½}$ was doubled in the presence of high DOM-SR and ASW (359 h) compared to DDW (156 h). The matrix affected glucose biodegradation but to a lesser extent ($P < 0.001$ ANCOVA); the fastest biodegradation occurred with DOM (6.6×10^{-3} h^{-1}) or in conjunction with ASW (8.0×10^{-3} h^{-1}).

Aislabie and Lloyd-Jones (1995) reported that adding organic material increases microbial activity accelerating pesticide degradation. Our data does not support this for Analar-PPT when DOM-SR was added as organic substrate ($t_{1/2}$ = 241 h). Qiao and Wang (2010) showed *Paracoccus* sp. KT-5 degraded glucose and pyridine simultaneously considering the degradation pathway to be metabolic while Xie et al. (2009) showed that short-chain fatty acids were favoured by *Acinetobacter johnsonii* MA19 over glucose as carbon sources for co-metabolism of malathion. The sediment here was sandy, low in OM (0.4% C) with low microbial sediment population (basal respiration = 24 µmol CO$_2$ kg^{-1} h^{-1}); possibly too low to observe significant effects. Also DOM-PPT interactions could have reduced PPT bioavailability; slowing PPT degradation but still in line with our sorption data suggesting DOM did not influence PPT sorption because DOM-PPT complexes would remain solvated.

Contrary to Analar-PPT biodegradation, glucose biodegradation was favoured by high DOM-SR (6.6×10^{-3} h^{-1}) and was fastest in high DOM-SR and low ASW (8.0×10^{-3} h^{-1}; half life 87 h)

suggesting the microbial population degrading glucose may be different to that degrading Analar-PPT (Table 4).

Slow Analar-PPT biodegradation rates in ASW (2.3×10^{-3} h^{-1}) and for the combined ASW and DOM-SR treatment (1.9×10^{-3} h^{-1}) could have been due to ASW effects on microbes from a freshwater site. DOM alone had a significant impact within 132 and 226 h (Wilcox procedure) (2.8×10^{-3} h^{-1}). Lay et al. (2010) reviewed salinity effects on membrane bioreactors illustrating that freshwater microbes do not possess mechanisms to cope with osmotic stress. However, Shiaris (1989) suggested that phenanthrene degraders in low salinity estuarine sediment subjected to saline were tolerant.

The data obtained for Analar-PPT biodegradation suggests that a mixture of metabolic and co-metabolic pathways were present, presumably because of the high diversity of microorganisms in the sediment. Singh and Walker (2006) reported that metabolic

(a)

(b)

Fig. 1. Effect of Zn (Panel a) and Pb (Panel b) on biodegradation of ^{14}C-glucose (Gluc) or Analar-PPT after 120 h and 240 h in high estuary sediment. Bars represent mean ± SEM ($n = 3$).

regulation depends strongly on what role the OP plays for the organisms involved.

Studying the effects of Zn and Pb on biodegradation, glucose biodegradation with Zn was not significantly affected at the times monitored ($P > 0.05$ ANOVA). At 120 h, Analar-PPT biodegradation (Fig. 1a) was affected at the lowest Zn concentration (2.2 µg g^{-1}), and this increased with increasing Zn, ($P < 0.05$ ANOVA and Tukey's pairwise comparison). After 240 h, PPT biodegradation was stimulated by 2.2 mg kg^{-1} Zn before dropping at 4.4 mg kg^{-1}. All Zn treatments differed from each other and the DDW control ($P < 0.05$ ANOVA and Tukey's pairwise comparison). The absence of Zn effects on glucose biodegradation suggests different microbes may metabolize glucose as most microbes do this. Soluble Zn was not detected after biodegradation suggesting it may have sorbed to sediment or precipitated if the local pH $\geqslant 7$ (Baird et al., 1997).

At 120 h, Pb effects on glucose biodegradation were minimal ($P > 0.05$ ANOVA) at the concentrations tested (Fig. 1b). However, after 240 h, a small increase in glucose biodegradation was seen at the highest Pb concentration ($P < 0.05$ ANOVA and Tukey's pairwise comparison) which was unexpected. This, and Pb reducing Analar-PPT biodegradation ($P < 0.05$ ANOVA), suggest only Pb exerts any toxic effect. As PPT was expected to exert toxic effects this suggests antagonistic toxicity with Pb (i.e. "cocktail" effect).

Analar-PPT biodegradation with Zn or Pb may be affected because microbes have to deal with more than one toxic compound so their response had to cope with both compounds, and PPT biodegradation could reduce in response to extra stress. Additionally, the PPT hydrolysing could be directly affected by Zn or Pb. Whilst Brown (1979) demonstrated that *Flavobacterium* sp phosphotriesterase activity was not affected by Zn^{2+}, this enzyme differs from phosphotriesterases in other microbes.

Table 4 shows reduced biodegradation rates for both PPT formulations with metal, DOM and salinity compared to the previous experiment. Glucose biodegradation was excluded from further analysis because statistical tests showed these data varied significantly from the PPT data. Fig. 1 shows metals reduced PPT biodegradation. Comparing Analar-PPT with PPT-Ind biodegradation with DOM and ASW, differences occurred when Zn and Pb were spiked in ASW (1066 h half life Analar-PPT; 912 h half life PPT-Ind), and for metals with DOM (1100 h half life Analar-PPT; 924 h half life PPT-Ind). Table 4 also shows that, with metals, $t_{1/2}$ was longer with ASW than without (e.g. 1066 h *versus* 737 h for Analar-PPT) suggesting low salinity may slow PPT biodegradation.

Comparing Pb and Zn data suggests that Zn affects PPT biodegradation less. Elevated Zn is well known in this river; for instance, river sediment pore water has been shown to contain significant Zn (1195 ± 862 µg L^{-1}) (García-Ortega, 2002), presumably microbes were adapted to Zn so PPT biodegradation was less affected. However, Pb reduced PPT degradation in all matrices. Lower Pb in river sediment (355 ± 256 µg L^{-1}) (García-Ortega, 2002) suggested microbes were less conditioned to Pb or the combination of Pb and PPT was more toxic for these microbes.

3.3. Microbial ecotoxicology

River sediment autochthonous microbes showed reduced CO$_2$ production over 60 min for both PPT formulations and in binary and ternary combinations with Zn and Pb (Table 5). Over 60 min, previous sorption studies have shown that >50% PPT should have remained dissolved and bioavailable with only a small proportion biodegraded (García-Ortega et al., 2006).

Results in Table 5 show that the toxicological microbial response to single compounds in ascending order, was Zn < Pb < Analar-PPT < PPT-Ind. A similar pattern was observed with medium DOM concentrations with slight evidence that DOM might reduce single compound toxicity. However, overlapping 95% confidence
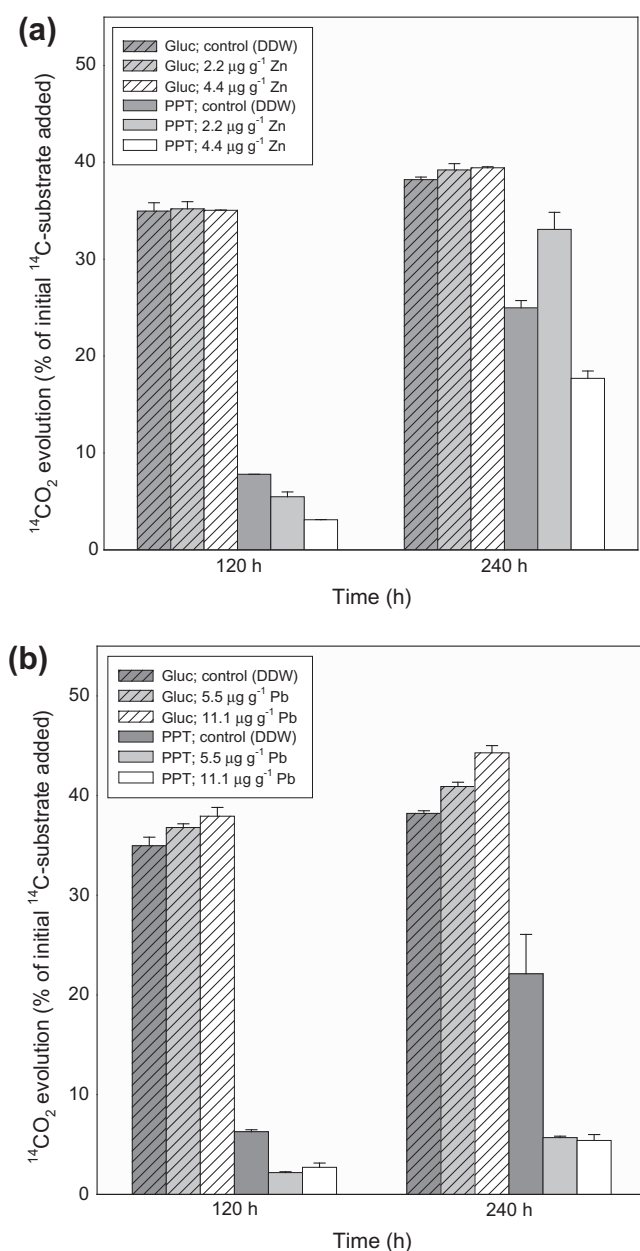
Table 5
Effective concentration (EC$_{50}$; µg g^{-1}) and Additive Index (AI) of river sediment microbes toxicological response in DDW with DOM* (40 mg C L^{-1}) for single, binary and ternary combinations of Analar-PPT, PPT-Ind with Zn or Pb. 95% confidence limits in brackets.

	Single compound toxicity – proportion ratio (1:100:10)				Binary compound toxicity – proportion ratio (1:100:10)			Ternary compound toxicity – proportion ratio (1:100:10)		
	PPT	PPT-Ind	Zn	Pb	PPT:Zn	PPT:Pb	Zn:Pb	PPT:Zn:Pb	PPT-Ind:Zn:Pb	PPT:Zn:Pb
EC$_{50}$	236 µg PPT g^{-1} (187–448)	14.0 µg PPT-Ind g^{-1} (6.6–30.9)	2127 µg Zn g^{-1} (1122–4467)	1445 µg Pb g^{-1} (447–10 000)	87.4 µg PPT g^{-1} (68.5–107.5)	93.3 µg PPT g^{-1} (83.3–101.6)	691.3 µg Zn g^{-1} (489.2–989.1)	34.6 µg PPT g^{-1} (28–41)	4.7 µg PPT-Ind g^{-1} (4.2–5.2)	0.52 µg PPT g^{-1} (0.46–0.61)
	404 µg PPT g^{-1*} (120–>1000)	23.0 µg PPT-Ind g^{-1*} (16.4–38.0)	5248 µg Zn g^{-1*} (1622–10 000)	2065 µg Pb g^{-1*} (371–10 000)	787.0 µg Zn g^{-1} (616.8–967.8)	570.9 µg Pb g^{-1} (509.5–621.5)	469.7 µg Pb g^{-1} (332.4–672.1)	312 µg Zn g^{-1} (255–369)	581 µg Zn g^{-1} (518–645)	472.0 µg Zn g^{-1} (415–555)
								212 µg Pb g^{-1} (173–250)	395 µg Pb g^{-1} (352–438)	32.1 µg Pb g^{-1} (28.2–37.7)
AI	–	–	–	–	0.35 (−0.44 to 2.40)	0.26 (−0.93 to 3.22)	0.54 (−1.40 to 3.22)	1.27 (−0.10 to 6.26)	0.22 (−1.34 to 2.48)	0.35 (0.71–9.34)

intervals suggest this was not statistically significant. This implies DOM does not affect PPT partitioning or bioavailability over 24 h suggesting interactions with DOM were weak reflecting that PPT is mildly hydrophobic (K_{ow} = 6600).

Van Beelen and Doelman (1997) reported four microbial resistance mechanisms to pollutants suggesting microorganisms that live with pollution withstand toxic stress by limiting uptake or maximizing pollutant excretion. Second, microorganisms acclimatised to a toxicant over long periods can induce phenotypical physiological resistance. Third, genetic resistance leading to competitive advantage can dominate after several generations. Fourth, resistant species replace sensitive ones forming "resistant" communities. One of these mechanisms has probably occurred here, not necessarily for PPT but possibly for Zn or Pb where previous exposure had occurred (García-Ortega, 2002; van Beelen and Doelman (1997) suggested elevated background concentrations are a common problem in risk assessment of natural chemicals because of difficulty in finding control samples without prior exposure.

Table 5 shows EC$_{50}$ data along with positive AI for all binary and ternary combinations although 95% confidence limits ranges do stretch to negative values. A positive or negative AI was expected for binary combinations (PPT:metal and Zn:Pb) because a change of toxic action was expected. In line with the positive AI for Zn:Pb, Parrot and Sprague (1993) found Cu and Zn were additive in their effect on bacterial luminescence and Chen and Yeh (1996) reported positive AI are frequent among chemicals with different toxic mechanisms, some being severely synergistic.

Table 5 shows ternary combinations toxicant effects were additive for Analar-PPT and PPT-Ind. PPT-Ind:Zn:Pb (4.7 µg PPT-Ind g^{-1}; 581 µg Zn g^{-1}; 395 µg Pb g^{-1}) was significantly more toxic than Analar-PPT:Zn:Pb (346 µg PPT g^{-1}; 312 µg Zn g^{-1}; 212 µg Pb g^{-1}) confirming industrial formulations should be considered when assessing pesticide toxicity.

An Analar-PPT:Zn:Pb ratio (1:100:10), which mimicked natural concentrations, showed an AI of 0.35 (calculated range 0.71–9.34) indicating a positive additive effect, with EC$_{50}$ of 0.52 µg PPT g^{-1}; 472.0 µg Zn g^{-1} and 32.1 µg Pb g^{-1} and EC$_{10}$ of 0.06 µg PPT g^{-1}; 51.0 µg Zn g^{-1} and 3.5 µg Pb g^{-1}. These data were for Analar-PPT, whilst previous data shows PPT-Ind was more toxic which is important given the UK Environment Agency Maximum Allowable Concentration (MAC) is 100 ng L^{-1} for PPT.

4. Conclusions

The data show that salinity and sediment–water chemistry affect PPT behaviour and toxicology more than DOM and that mass transfer and partitioning slow PPT sorption but that, once sorbed, PPT-sediment binding is strong mainly influenced by organic matter but less so by salinity. Slow PPT sorption and delayed effects of DOM on PPT sorption are important in a dynamic river system because PPT travelling downstream would not reach equilibrium before passing through several cycles of sorption (decreasing concentration, toxicant effects and biodegradation) and desorption (extending toxic effects). Equally, salinity increasing PPT sorption means dissolved PPT might not reach the open sea although PPT bound to DOM or sorbed on sediment carried to the sea means that, after a tidal cycle, PPT might desorb with the incoming tide and move back up the estuary. Analar-PPT and PPT-Ind sorption differences with salinity are important because they can influence bioavailability and toxicity and ascribed to solvent in the latter.

Evidence suggests some solution PPT exists as a PPT-DOM complex altering interactions with microbes and varying PPT biodegradation down the estuary. Thus, whilst Zn and Pb reduce PPT biodegradation, DOM reduces this effect whilst salinity or salinity

with DOM both appear to induce osmotic shock on the microbes reducing Analar-PPT and glucose biodegradation.

Binary and ternary combinations of Analar-PPT with metals were additive in equitoxic ratios implying these do not interact strongly during their action. DOM slightly decreased toxicity implying DOM-metal interactions do not affect bioavailability. Mimicking pore water values with Analar-PPT–Zn–Pb (ratio 1:100:10) showed an EC_{10} for Analar-PPT below the UK Environmental Agency MAC suggesting this should consider PPT-Ind toxicity in combination with other pollutants (e.g. metals). Further work would integrate this knowledge into risk assessment models to improve predictive capabilities.

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