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Toxicology and fate of Pestanal® and commercial propetamphos formulations in river and estuarine sediment

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Abstract

To quantify the impact of organophosphate pesticides on aquatic ecosystems requires a mechanistic understanding of their behaviour in a range of environmental matrices. The objective of this study was to compare the sorption/desorption, biodegradation and toxic effects of the Pestanal® grade and commercial formulation (Ectomort Centenary) of the organophosphate insecticide propetamphos in river and estuarine sediments. For both formulations, the sorption of propetamphos onto sediment was initially very rapid followed by a slower sorption phase. Similarly, the initial rate of desorption was rapid, followed by a much slower rate. In both sorption and desorption experiments, the level of sorbed propetamphos was considerably higher for the commercial formulation of propetamphos (K_d=7–11) than for the Pestanal® grade (K_d=4–10). The rate of propetamphos biodegradation was sediment dependent but was most rapid where microbial activity and nutrients were the highest and sorption was the lowest. Propetamphos was more rapidly degraded in sediments under aerobic ($t_{1/2}$=15 d) compared to anaerobic conditions ($t_{1/2}$=19 d). However, no significant difference in the biodegradation rates of the Pestanal® grade and commercial formulations of propetamphos were observed. The toxic effect of propetamphos on sediment microbial communities was significantly greater for the commercial formulation than for the Pestanal® grade of propetamphos based on EC_{50} (21 versus 236 μg g^{-1}) and EC_{10} values (0.3 versus 54 μg g^{-1}). In conclusion, our results highlight the importance of using commercial pesticide formulations when carrying out ecotoxicological testing.

Keywords: Bioavailability; Fate; Pesticide formulation; Sheep dip; Safrotin; Toxicology

1. Introduction

Since the discovery of agrochemical compounds in apparently unpolluted ecosystems, the factors that control their transport, persistence and toxicology in a range of environments have been intensively studied. Jensen et al. (2004) have studied the interactions

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between sorption and biodegradation processes, rather than these processes in isolation to determine pesticide persistence and mobility. The kinetics and mechanisms of sorption and desorption have also been linked with mobility, volatilisation and degradation (Farrell and Reinhard, 1994). More recently, it has been stated that pesticide sorption and desorption data are imperative in the prediction of xenobiotic attenuation and bioavailability in ecosystems (Gao et al., 1998; Lawrence et al., 2000). Collectively these researchers have stressed that transport, persistence and toxicology are intrinsically linked and therefore needed to be studied rather than in isolation.

Propetamphos (the subject of this study) is a non-ionic organophosphorus pesticide used for ectoparasite control in sheep and for the control of household and public health pests (e.g. cockroaches, mosquitoes; USEPA, 2000). Many incidents of aquatic environment pollution by propetamphos (<0.4 μg l^{-1}) along with serious incidents (1 μg l^{-1} to 1 mg l^{-1}) have been reported due either to poor placement of sheep dipping sites or unregulated disposal of spent dip (Environment Agency, 2002; Virtue and Clayton, 1997). Propetamphos is persistent in aqueous environments as abiotic hydrolysis is slow even in the presence of sunlight ($t_{1/2}$>1 y; Tomlin, 1994). It has an octanol/water partition coefficient (K_{OW}) of 6600 ($\log_{10} K_{OW}$=3.82) and possesses low aqueous solubility (110 mg l^{-1} at 24 °C; Tomlin, 1994). Propetamphos is primarily available in two forms; the pure, Pestanal® grade compound and as a commercial formulation (e.g. Ectomort Centenary, Young's Summer Dip, Safrotin, Zoecon). Generally, published studies on pesticides have tended to focus on the Pestanal® grade of pesticides. However, the commercial formulations can be chemically different particularly in terms of the solubility of the active pesticide which, in turn, would be expected to affect sorption, biodegradation and toxicity properties. In terms of its toxicity, propetamphos, whilst highly effective as an insecticide, has been reported to be particularly toxic to aquatic invertebrates with EC_{50} concentrations for sensitive species such as water fleas and insect larvae in the 1 to 100 μg l^{-1} concentration range (PAN, 2004; Gälli et al., 1994). EC_{50} values for fish, zooplankton, phytoplankton and annelida typically range from 250 to >10,000 μg l^{-1} (PAN, 2004).

In the study reported here, sediment microbial respiration has been used to measure propetamphos toxicity. Microbes represent the lowest trophic level in sediment, mineralizing dead biomass and organic matter. Due to their ubiquitous presence, it has been reported that microbes can act as a very relevant indicator of environmental pollution (van Beelen and Doelman, 1997). For instance, Eismann and Montuelle (1999) stated that a change in microbial community structure in sediment has consequences for the higher trophic levels and for the environmental status of the overlying water column. These researchers also stated that, in the case of microbial studies, metabolic parameters such as the uptake or conversion of ubiquitous substrates in aquatic environments (e.g. glucose, amino acids) represent the best way of measuring toxic effects.

The first aim of this paper was to present a combined study of the fate, biodegradation and microbial toxicology of the insecticide propetamphos in river and estuarine sediments under aerobic and anaerobic conditions. The second aim was to compare the behaviour of the Pestanal® grade and commercial formulation (Ectomort Centenary) of propetamphos.

2. Materials and methods

2.1. Sampling and sample treatment

Sediment samples (ca. 1 kg per site) were taken from three contrasting sites along the Conwy River, North Wales, UK in January and August 2000 and May 2001. The river is located in a predominantly sheep grazed catchment area (344 km^2) where frequent dipping of livestock occurs and has a mean annual freshwater discharge of 20 m^3 s^{-1} (range 0.5 to 500 m^3 s^{-1}). Sediment 1 ('LL') was collected from a non-tidal stretch of the river at Llanrwst (53°08′ N 3°50′ W) located 22 km from the estuary mouth. Sediments 2 and 3 were taken from the river estuary on the outgoing tide from recently exposed sediment. Sample 2 was in the upper tidal estuary ('TC') at Taly-Cafn (53°13′ N 3°48′ W) 11 km from the estuary mouth whilst sample 3 was taken from the lower estuary ('BS') at the Conwy RSPB Nature Reserve (53°17′ N 3°48′ W) 2 km from the estuary mouth. Sediment samples were collected as intact cores (10

cm diameter, 0–10 cm depth) with a high-density polyethylene corer. At each site, a series of replicate cores was bulked and the samples were transported at 5 °C to the laboratory within 6 h of collection where they were also stored at 5 °C.

2.2. Sediment analysis

Sediment pH and electrical conductivity were measured according to the procedure described in Page et al. (1982). After sediment pore water removal by centrifugation, NH_4^+ was determined colorimetrically by the indophenol blue method (Page et al., 1982), NO_3^- colorimetrically after hydrazine reduction to NO_2^- (Downes, 1978) and P colorimetrically as described in Murphy and Riley (1962). Sediment carbon content was analysed using a CHN-2000 Analyzer (Leco Corp, St Joseph, MI). CO_2 evolution (basal respiration) was used as a measure of total sediment biological activity and was determined using a CIRAS-IRGA-SR1 respirometer (PP Systems Ltd., Hitchin, UK). Sediment particle size was determined by wet sieving with data analysed according to Hodgson (1976) and Day (1967). For sediment analysis, standard errors in all cases were less than 5% of the mean value.

2.3. Chemicals

Propetamphos (1-methylethyl (*e*)-3-{{(ethylamino)methoxyphosphinothioyl}oxy}-2=butenoate) was purchased as the Pestanal® grade chemical (95.9% purity; Sigma-Aldrich Ltd., Poole, UK) and as a commercial formulation known as Ectomort Centenary (8%, Vericore Ltd., Dundee, UK). Radiolabelled ^{14}C-propetamphos was also used (0.732 MBq kg^{-1}; radiochemical purity 97.9%; Huntingdon Life Sciences Ltd., Alconbury, UK). All other chemicals were Analar grade.

2.4. Sorption and desorption experiments

Experiments were carried out in Pyrex® glass bottles (25 ml) with Teflon® liners to prevent propetamphos adsorption to the container. Sediment samples (four replicates per sediment) from LL, TC and BS were tested using Pestanal® grade or commercial formulation propetamphos concentrations of 1, 10, 25 and 75 mg l^{-1}. Controls containing propetamphos but no sediment were also included. Briefly, 2 g of sediment was shaken with 20 ml of ^{14}C-labelled propetamphos solution (0.09 kBq ml^{-1}) at 150 rev min^{-1} in the dark at 20 °C for up to 24 h. NaN_3 was added to the solutions (40 mM) to minimize microbial activity. After shaking for 0.08, 2, 4, 8 and 24 h, the sediments were centrifuged at 14,000 $\times g$ for 5 min and the supernatant retained. The amount of ^{14}C-labelled propetamphos remaining in the supernatant (equilibrium solution concentration; C_{sol}, μg cm^{-3}) was determined by liquid scintillation counting on a Wallac 1409 (Perkin-Elmer Life and Analytical Sciences, Boston, MA). The amount of sorption (C_{ads}, μg g^{-1}) was calculated by difference from the amount initially added and that recovered at each harvest time. Sorption was expressed on a sediment dry weight basis. The Freundlich sorption isotherm equation was fitted to the experimental data by a computerized least squares optimisation routine where

$$C_{ads} = K_f \times C_{sol}^n \quad (1)$$

and where K_f and n are coefficients (Fytianos et al., 2000). The solid-to-solution partition coefficient (K_d) was determined from the equation

$$K_d = C_{tot}/C_{sol} \quad \text{where} \quad C_{tot} = (C_{sol} \times \theta) + (C_{ads} \times \gamma)$$

where C_{tot} is the total propetamphos in the sediment (mg cm^{-3}), θ is moisture content (cm^3 cm^{-3}) and γ is the bulk density (g cm^{-3}) (Barker, 1995).

Propetamphos desorption was determined after the last sorption measurement had been made (i.e. after 24 h). After a sediment equilibration period of 24 h with the ^{14}C-labelled propetamphos, the sediment and supernatant were centrifuged at 9000 $\times g$ for 10 min. The supernatant was then removed and replaced with distilled water (20 ml) containing 40 mM NaN_3 and the samples shaken in the dark for 1, 24 and 48 h as described above. After 1, 24 and 48 h, solution ^{14}C-labelled propetamphos, and consequently the amount of desorption, was determined by liquid scintillation counting as described above. The propetamphos which remained sorbed to the sediment was calculated as the difference between the amount desorbed at each harvest time subtracted from the amount sorbed at 24 h. Desorption isotherms were calculated using a linear model.

2.5. Biodegradation of propetamphos

Wet sediment (2 g dry weight) was placed in a 100 ml Pyrex® glass bottle and spiked with 2 ml of ^{14}C-labelled propetamphos (0.17 kBq ml^{-1}) and Pestanal® grade propetamphos to give a sediment concentration of 0.685 mg kg^{-1}. After hermetically sealing the bottles, the $^{14}CO_2$ evolved over time was captured using a 1 M NaOH trap suspended above the sediment. The samples were incubated at 20 °C under low light intensity conditions (75 μmol quanta m^{-2} s^{-1}). One set of bottles was incubated under aerobic conditions whilst the other set of bottles was maintained under anaerobic conditions by daily purging the bottles with N_2 gas for 30 min. The amount of $^{14}CO_2$ in the NaOH traps was determined over a 32 d period by liquid scintillation counting as described above. Control samples containing propetamphos but no sediment were also performed. Individual treatments had four replicates.

2.6. Propetamphos toxicity

Toxicity experiments were developed from a method described by van Beelen and Doelman (1997). Specifically, Pestanal® grade or commercial formulation (Ectomort Centenary) propetamphos was added to sediment as described above to give concentrations of 0.1, 1, 10, 60, 110, 140, 500 and 1000 mg kg^{-1} sediment. Uniformly ^{14}C-labelled glucose (Sigma-Aldrich Ltd.; 9.25 MBq mol^{-1}) was then added to the sediment to give a final concentration of 45 μg g^{-1} and $^{14}CO_2$ evolution determined as described above (after 1 h). Individual treatments had four replicates. Effective concentrations were determined as the propetamphos concentration causing either a 50% (EC_{50}) or 10% (EC_{10}) reduction in microbial respiration (measured as $^{14}CO_2$ production) in comparison with the control (unamended sample; Nirmalakhandan et al., 1994).

3. Results and discussion

3.1. Sediment characteristics

A summary of the characteristics of the three sediments used in this study is shown in Table 1. All the sediments possessed a predominantly sandy texture and had chemical properties reflecting their position in either the tidal or non-tidal stretches of the river (i.e. salinity). All the sediments had a redox potential of around 170 mV indicating subtoxic conditions.

3.2. Propetamphos sorption and desorption

The sorption kinetics of Pestanal® grade propetamphos on the three sediments are shown in Fig. 1a. In all cases, the kinetics were characterised by an initial rapid phase of sorption (<5 min) followed by a slower sorption phase (2 to 4 h) with the system appearing to reach a quasi-equilibrium after 8 h. These results are in general agreement with previous reports on organophosphate pesticide sorption to soils and sediments. For example, in freshwater sediment, demeton-*s*-methyl, methidathion, azinphos-methyl and phosalone all appeared to reach sorption equilibrium within 5 h (Fröbe et al., 1989). In general, sediment 1 (LL) possessed the highest sorption capacity with a solid-to-liquid partition coefficient (K_d) of 11 at equilibrium. In contrast, sediments 2 (TC) and 3 (BS) had lower sorption capacities with K_d values of 2.7 and 2.2, respectively. Although the number of sediments tested here is limited, sorption was highest in the sediment with the highest C content (Table 1). Interestingly, Cooke et al. (2004) showed propetam-

Table 1
Selected chemical, biological and physical characteristics of the three Conwy River sediments

Sediment	Moisture content (g kg^{-1})	pH	Salinity (g kg^{-1})	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})	P (mg kg^{-1})	Total C (g kg^{-1})	Basal respiration (g CO_2 kg^{-1} h^{-1})	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})
1. LL	425	5.73	1.1	2.8	77	21	34	1.0	715	140	60
2. TC	272	8.06	17.7	2.8	1	59	12	5.1	694	172	105
3. BS	255	8.35	28.3	5.6	1	99	12	5.5	928	13	29

All values are expressed on a dry weight basis. Values represent means (n=3).

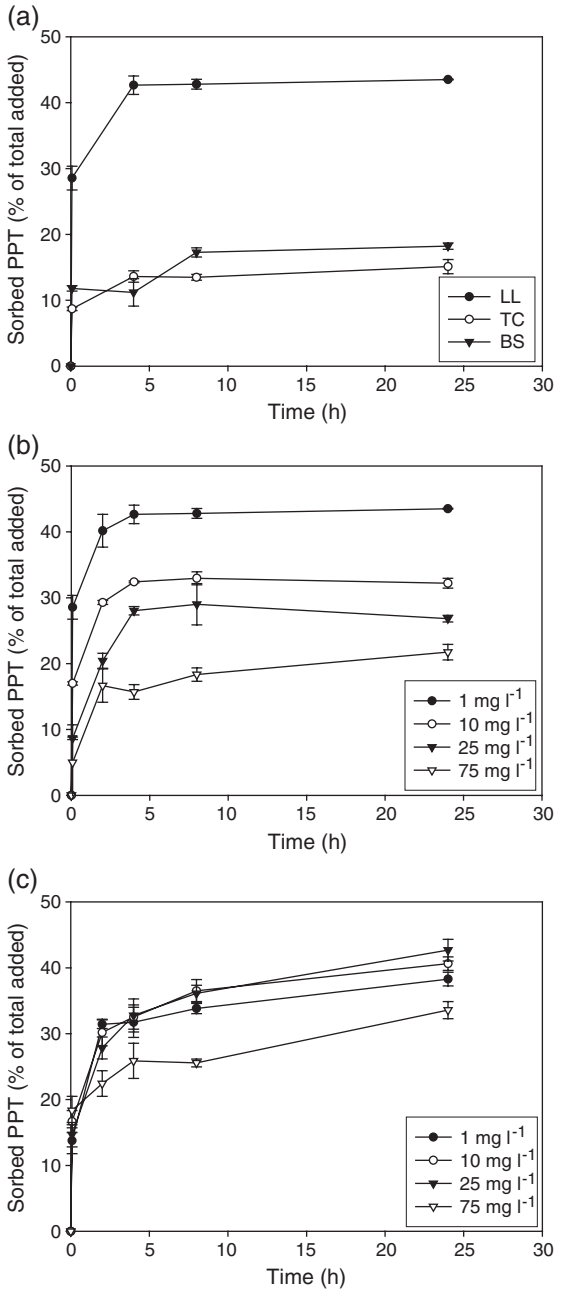

Fig. 1. Sorption kinetics of (a) Pestanal® grade propetamphos (1.0 mg l^{-1}) onto three sediments from the Conwy River and Estuary, (b) varying concentrations of Pestanal® grade propetamphos onto LL sediment and (c) varying concentrations of commercial grade propetamphos onto LL sediment. Values represent mean ± standard error of the mean (SEM), $n=3$.

phos sorption in soil to be linked to humic substance content more closely than total C. By comparison, our data showed no correlation between the degree of propetamphos sorption and clay content (Table 1). Across the three sediments, the amount of propetamphos sorption was low in comparison to previous studies on other organophosphorus pesticides (10% to 30% of the propetamphos was sorbed). For example, 80% of methyl parathion and 60% of malathion was sorbed onto estuarine sediments after 6 h (Sujatha and Chacko, 1992).

Fig. 1 also shows the sorption kinetics of Pestanal® grade and the commercial formulation of propetamphos, onto the LL sediment for a range of added concentrations. For the Pestanal® grade propetamphos (Fig. 1b) our results show that, as the applied pesticide concentration increases, the percentage of sorbed material drops indicating a saturation of sorption sites at higher applied concentrations. In agreement with Fig. 1a, the sorption reaction for all concentrations was complete after 8 h. In contrast, the kinetics of propetamphos sorption when applied in the commercial formulation (Fig. 1c) were significantly different from those when applied as the Pestanal® grade ($P<0.01$). Generally, the propetamphos took longer to equilibrate with the sediment with sorption still occurring 24 h after addition (Fig. 1c). At low propetamphos concentrations, proportionally more Pestanal® grade was sorbed in comparison to that in the commercial formulation, however, this trend was reversed at higher concentrations. Across the concentration range studied (1 to 75 mg l^{-1}), the K_d values for the Pestanal® grade propetamphos ranged from 4 to 10 whilst the K_d values for the commercial formulation were similar and ranged from 7 to 11. The difference observed between the Pestanal® grade and commercial formulation of propetamphos may indicate that sorption to different sites may take place in the presence of the organic solvent (Shellsol R®) in the commercial formulation.

The desorption kinetics for both the Pestanal® and commercial formulations showed a very similar pattern with a fast desorption phase in the first hour after which the rate of desorption reached a new equilibrium (Fig. 2). However, for the commercial formulation, almost twice as much pesticide remained sorbed at the highest initial concentration (75 mg l^{-1}). This situation correlates with the sorption data where a

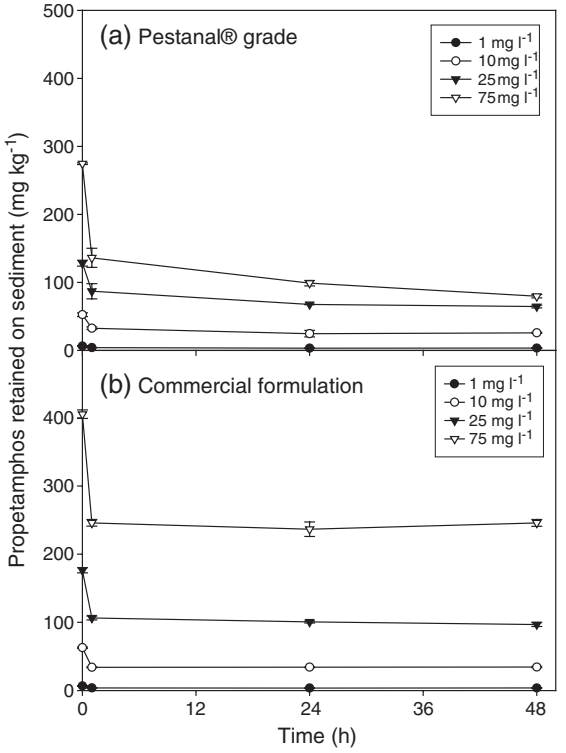

Fig. 2. Desorption kinetics of (a) varying concentrations of Pestanal® grade propetamphos and (b) varying concentrations of commercial grade propetamphos from LL sediment. Values represent mean ± standard error of the mean (SEM), ($n=3$).

higher proportion of propetamphos was sorbed at higher concentrations for the commercial formulation. This tends to suggest that, as for the sorption data, different sorption sites are accessible to the commercial formulation with a much greater affinity for propetamphos. Farrel and Reinhard (1994) showed that none of the physically measurable properties of the solid phase (e.g. size fraction) had any correlation with the amount of slow desorbing trichloroethylene from model sediments. Cornelissen et al. (1998) studied the desorption kinetics of non-ionic compounds (PCBs and chlorobenzenes) from sediment and concluded that, although both mineral micropores and organic matter can contribute to slow desorption, processes occurring in the organic matter dominate over processes in the mineral matrix, when organic matter content is 0.1% to 0.5%. In the present work, total C was similar for BS and TC but higher for LL with the latter retaining more propetamphos after desorption suggesting a similar link between these parameters.

Fig. 3 shows the 24 h sorption data for Pestanal® and commercial grade propetamphos on LL sediment with the regression lines showing good correlation to the Freundlich sorption isotherm ($r^2>0.99$). The data show clearly the higher uptake of the commercial formulation over the Pestanal® grade. This situation was also observed for the BS sediment, although here less Pestanal® grade pesticide was sorbed as occurred for the TC sediment (e.g. after 24 h with 75 mg 1^{-1} propetamphos, LL sorbed 22% Pestanal® and 34% commercial grade whilst BS sorbed 4% and 34%, respectively). As stated previously, sorption correlates well with C content (Table 1). Thus, it appears that the aromatic solvent (Shellsol R®) present in the commercial formulation enhances propetamphos sorption onto sediment organic matter.

3.3. Biodegradation of propetamphos

It should be noted that the abiotic hydrolysis of propetamphos is extremely slow compared with the timescale of these experiments (Tomlin, 1994). Therefore, it can be concluded that the $^{14}CO_2$ produced in these experiments was predominantly due to propetamphos biodegradation by the indigenous microbial population and not chemical hydrolysis. In this respect, it has been reported that organophosphorus pesticides undergo hydrolysis by a number of differ-

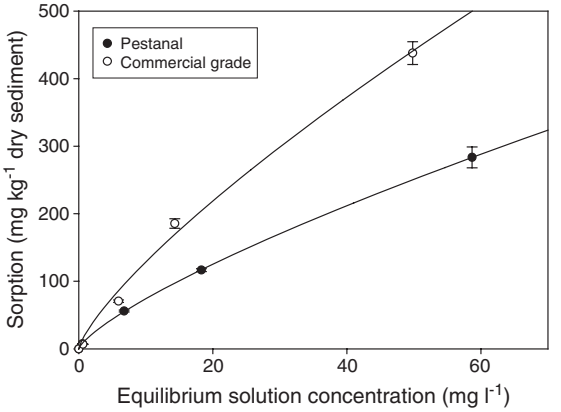

Fig. 3. Sorption of Pestanal® and commercial grade propetamphos to the LL sediment after 24 h equilibration. Points represent the averaged experimental data and lines represent regression fits of the Freundlich isotherm ($r^2>0.99$).

ent enzymatic processes (Sogorb and Vilanova, 2002) such that hydrolysis can occur at the ester or acid anhydride bond (Wyman and Ballard, 1982). Phosphate is produced from this hydrolysis along with and organic byproducts which further degrade to CO_2 (the end point of these studies) and water.

No significant differences in the biodegradation rates of the Pestanal® grade and commercial formulations of propetamphos were observed in our studies ($P>0.01$ GLM) implying that the two formulations were biodegraded by the same processes. On this basis, the following data can be considered to apply to both formulations.

Fig. 4 shows data for the biodegradation of ^{14}C-propetamphos under aerobic and anaerobic conditions presented as the amount of $^{14}C–CO_2$ evolved on a daily basis. Both aerobic and anaerobic data indicate a different pattern of degradation between the three sediments with the order of fastest to slowest being BS>LL>TC. Sediments from LL and TC seem to have a "conditioning" time (lag phase), which is not apparent in the BS sediment. The lag phase may indicate that the microbial population was only able to degrade propetamphos after some "conditioning" to the new aqueous chemistry. In contrast, biodegradation of propetamphos in the BS sediment was rapid and almost immediate. This may indicate that the BS sediment may have had previous pesticide exposure.

The aerobic and anaerobic biodegradation data shown in Fig. 4 were also converted to cumulative percentage degradation per gram of sediment (data not presented). The cumulative data show that the maximum evolution of $^{14}C–CO_2$ under an anaerobic atmosphere was only ca. three quarters of the value under aerobic conditions. This indicated slower initial degradation under anaerobic conditions although the total propetamphos degraded after 35 days was similar for both aerobic and anaerobic conditions in all sediments. This is reflected in the longer half-lives for anaerobic atmosphere (Table 2) suggesting that the microbial population was facultative and had to acclimatise to anoxic conditions. This is in line with previous reports of diazinon hydrolysis by *Flavobacterium* sp. being more rapid under aerobic conditions (Sethunathan and Yoshida, 1973).When it is considered that aerobic conditions often below the top 2 cm of sediment (ERASM, 1999), if propetamphos passes below the top 2 cm layer, the biodegradation rate may slow presenting a greater toxic risk to the environment. This is perhaps most relevant in the TC sediment where sorption is lower (than LL for instance) so propetamphos transport below the top 2 cm layer may be more likely but where a propetamphos degradation lag phase is observed.

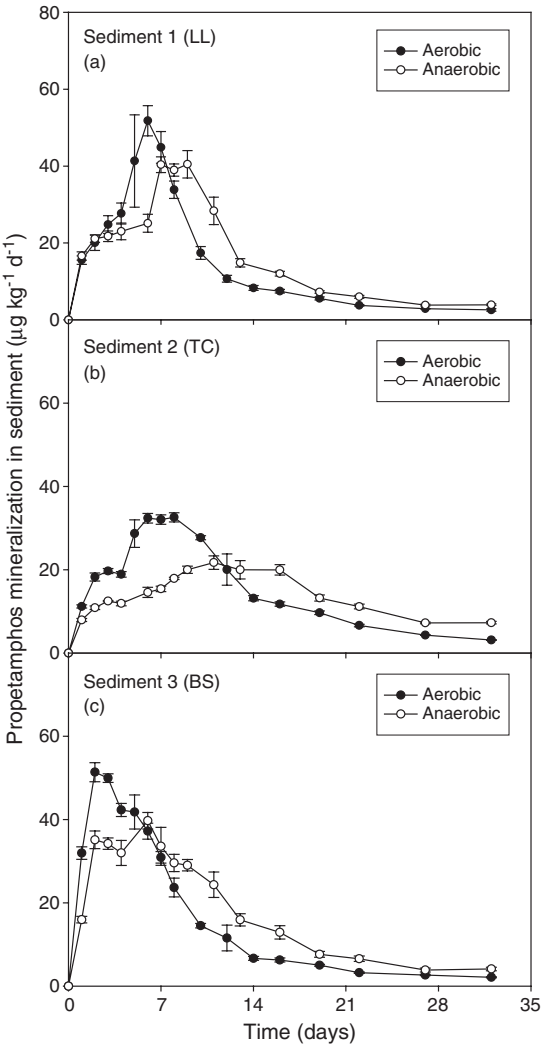

Fig. 4. Evolution of $^{14}C–CO_2$ per day from ^{14}C-propetamphos mineralization in (a) LL, (b) TC and (c) BS sediments from the Conwy River under aerobic and anaerobic conditions. The initial propetamphos concentration was 0.685 µg g^{-1}. Values represent mean ± standard error of the mean (SEM), ($n=3$).

The cumulative graphs have been used to analyse the biodegradation kinetics using a semi-logarithmic linear biodegradation model. Table 2 shows values for the theoretical half-life (DT_{50}) calculated using this

Table 2
Calculated (DT_{50}) and experimental ($t_{1/2}$) values from the sediment biodegradation of propetamphos under aerobic and anaerobic conditions

Sediment	Equation ($\log_{10}$ C/C_o=)	Aerobic				Equation ($\log_{10}$ C/C_o=)	Anaerobic			
		S	r^2	DT_{50} (h)	$t_{1/2}$ (h)		S	r^2	DT_{50} (h)	$t_{1/2}$ (h)
LL	−0.051 −0.00066 t	0.057	0.86	377	270	−0.024 −0.00063 t	0.041	0.93	442	345
TC	−0.011 −0.00072 t	0.038	0.94	404	330	−0.021 −0.00054 t	0.013	0.99	523	580
BS	−0.110 −0.00065 t	0.063	0.84	295	205	−0.042 −0.00069 t	0.044	0.93	373	290
Mean (SEM)				359 (32)	268 (38)				446 (43)	405 (89)

DT_{50} values were calculated using a semi-logarithmic kinetic model. S=standard deviation of the curve and r^2 =correlation coefficient. Data collected under laboratory light conditions, 20 °C and pH 5.7–8.3.

model along with the experimental half-lives ($t_{1/2}$) determined from the graphs. Firstly, comparing the DT_{50} and $t_{1/2}$ values from the three sediments, the degradation rate of propetamphos under aerobic conditions in the LL and TC sediments was significantly slower than in the BS sediment ($P<0.001$, GLM). Comparing these degradation rates with the physicochemical sediment characteristics (Table 1), the faster rates in the BS sediment correlate with the fact that this sediment has the highest microbial respiration (5.5 g CO_2 kg^{-1} h^{-1}) and inorganic nutrients (P 99 mg kg^{-1}; NH_4^+–N 5.6 mg kg^{-1} and salinity 28.3 mg kg^{-1}). The TC sediment showed a slower rate of degradation than the LL sediment despite showing greater microbial respiration activity and inorganic nutrient values greater than the LL sediment (Table 1). This behaviour was also despite the fact that the LL sediment had greater propetamphos sorption behaviour than the TC sediment which might have been expected to reduce the bioavailability of the pesticide. Instead, the slower rate is ascribed to a combination of the fact that the TC sediment exhibited longer lag times before reaching maximum CO_2 evolution along with the lowest CO_2 evolution at this point (Fig. 4) which might imply that this site had less history of pollution than the others (see discussion above).

Table 2 shows that the maximum theoretical half-life (DT_{50}) is for the TC sediment (ca. 21 days). However, Table 2 also shows that most DT_{50} values appear to be overestimated compared with the experimentally derived $t_{1/2}$ values although all the fits to the model are adequate ($r^2>0.84$). The average half-life (DT_{50}) for the three sediments under aerobic conditions is 359 h (ca. 15 days) compared with 446 h (ca. 19 days) under anaerobic conditions. These are typical values for an organophosphorus pesticide. For instance, Cotham Jr. and Bidleman (1989) reported that the half-life of endosulfan I in seawater/sediment system was 22 days and fenvalerate 12 days (20 °C, pH 7.3–7.7) whilst Liu et al., 2001 reported chlorpyrifos hydrolysis half-lives varying from 24 to 124 days in the Chesapeake Bay region.

3.4. Microbial ecotoxicology

These experiments were designed to measure the physiological response of the indigenous microbial population of LL sediment (measured as $^{14}CO_2$-production from ^{14}C-glucose substrate) following the addition of either Pestanal® grade or the commercial formulation of propetamphos. Following preliminary experiments, an exposure time of 60 min was chosen knowing that indigenous microbial populations respond rapidly to environmental change, both in terms of metabolic rate and, to a smaller extent, community composition (Burton, 1991). This time period was a compromise because it minimised any influence of biodegradation (loss of propetamphos during the first day of incubation in LL sediment was only ca. 2%) and because enhanced microbial growth due to the addition of glucose substrate would not have been expected to occur. However, the system would not be expected to reach chemical equilibrium in this time.

Fig. 5 shows that the microbial population has a certain resistance to low concentrations (<10 mg kg^{-1}) of Pestanal® grade propetamphos but that above this the evolution of ^{14}C–CO_2 drops considerably. By comparison, the glucose mineralization data when the commercial grade of propetamphos was applied show a different, sigmoidal pattern (Fig. 5) with a 20% reduction in ^{14}C–CO_2 production compared to Pestanal® grade propetamphos observed up

to 1 mg kg^{-1}, then a drop in $^{14}C–CO_2$ production up to 100 mg kg^{-1} with stabilisation above this concentration. These toxic effects are observed even though the main toxic effect of organophosphates is well known to be the inhibition of acetyl cholinesterase activity. However, Gälli et al. (1994) have reported that for some organophosphorus pesticides (e.g. fenitrothion) toxic effects were not directly correlated to the inhibition of acetyl cholinesterase but instead that metabolic activation of the pesticide could be responsible.

The empirical equations for toxicology fitted to all data gave good correlations ($r^2 > 0.88$). Calculations of EC_{50} values (Table 3) show considerably higher values for the Pestanal® grade propetamphos (236 μg g^{-1}) compared with the commercial grade (21 μg g^{-1}), indicating that the commercial formulation of the pesticide induced a 10-fold increase in the toxic response compared with the pure pesticide.

However, EC_{10} values are considered to be more important for decision making than EC_{50}, because these values indicate the levels of pollution that a specific community can tolerate without suffering drastic ecological changes. For instance, it has been reported that a 10% inhibition of a process can be accompanied by more than 50% inhibition of the most sensitive species (van Beelen and Fleuren-Kemilä, 1999) and it has been suggested by van Beelen and Doelman (1997) that resistant microorganisms often fail to perform specific ecological functions, thus ecological impact can be even greater.

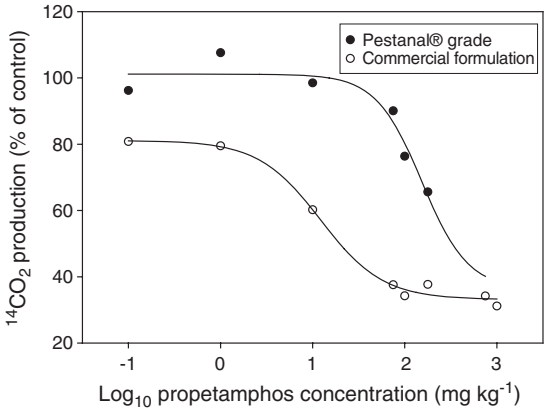

Fig. 5. $^{14}C–CO_2$ production as percent of control (no propetamphos added) after 60 min for Pestanal® and commercial grade propetamphos. Curves show the best fit to theoretical equations.

Table 3
EC_{50} and EC_{10} values for Pestanal® grade and a commercial formulation (Ectomort Centenary) of propetamphos applied to LL sediment in a distilled water matrix

Formulation	EC_{50} (μg g^{-1})	EC_{10} (μg g^{-1})
Pestanal® grade	236 ± 9	53.7 ± 2.0
Commercial formulation	20.5 ± 1.0	0.33 ± 0.02

Values represent means ± SEM ($n = 3$).

In line with the EC_{50} values, the EC_{10} data (Table 3) again show higher values for the Pestanal® grade propetamphos (54 μg g^{-1}) compared with the commercial grade (0.3 μg g^{-1}). However, in this instance the difference between the formulations is more pronounced with the commercial formulation being considerably more toxic. This is ascribed to the presence of a solvent (Shellsol R®). This might be expected to increase the solubility and bioavailability of the pesticide although our data do not support this by showing a higher affinity for the sediment phase for the commercial formulation and no differences between the biodegradation of the two formulations. Secondly, and in line with our data, the solvent itself may exert a toxic effect either by itself or in combination with the propetamphos. The significantly higher toxicant effect measured for the commercial formulation confirms the importance of assessing commercial pesticide formulations as the use of pure pesticide may mask their true ecological behaviour and fate.

4. Conclusions

Three parameters (sorption/desorption, biodegradation and toxicity) have been measured for two formulations of propetamphos. In the biodegradation tests, no significant differences are observed between the two pesticide forms suggesting the Shellsol R® solvent present in the commercial formulation does not effect this process. Differences were observed for the sorption, and to a smaller extent on desorption, with the commercial formulation showing a greater affinity for the sediment phase suggesting the Shellsol R® solvent enhances access of the pesticide to sorption sites not available to the Pestanal® formulation. However the most significant differences are seen in the toxicity data with the commercial formulation

showing significantly greater microbial toxicity from both the EC_{50} and EC_{10} data.

The different responses of the Pestanal® and commercial grade formulations to the sorption/desorption, biodegradation and toxicity tests suggest that it is not safe to extrapolate from one set of data to another to compare pesticide formulations. Clearly the data also show the importance of testing commercial pesticide formulations as these are the formulations released into the environment. This has important considerations because environmental policy is usually based upon data from Pestanal® grade pesticides when, in this instance, the commercial formulation appears to be more toxic.

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