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Dissipation, Distribution, and Uptake of ^{14}C-Chlorpyrifos in a Model Tropical Seawater/Sediment/Fish Ecosystem

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Chlorpyrifos, O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate, is one of the most widely used organophosphorous pesticides worldwide due its high efficacy (Carvalho et al. 1992; Liu et al. 2001; Meikle and Youngson 1978). In Kenya, Dursban formulations are mainly used for protection of horticultural fruits and vegetables (Lalah 1994). Because of its low water solubility (0.4 mg/L) and high hydrophobicity (log K_{ow} of 5.27), it is believed that chlorpyrifos would be able to partition easily onto aquatic sediments and macrophytes where it can pose dangers to benthic organisms (Ronday et al. 1998). It is also quite a volatile toxicant due to its low vapour pressure (2.5 mPa at 25 °C) and low air-to-water partition coefficient (8.9 10^{-4} at 25 °C) and its residues have been detected in air and rainwater samples (Liu et al. 2001).

Increasing use of chlorpyrifos also causes a lot of anxiety to environmentalists and toxicologists because it is toxic to both humans and wildlife. As an irreversible inhibitor of acetylcholinesterase, it can cause impairment in mammalian brain cell development (Lund et al. 2000; Jeanty et al. 2001; Jett et al. 1999; Slotkin et al. 2001). Widespread use of this compound is therefore considered to be of great danger particularly to pregnant women and children. Some of its reported toxicities to aquatic organisms include 96 hr LC_{50} of 0.13 µg/L and 96 hr LC_{50} of 0.035 µg/L in adult *Neomysis integer* and *Americamysis bahia*, respectively (Roast et al. 1999). Although its toxicity in mammalian and aquatic organisms has been well documented, its fate and effects on aquatic ecosystems in tropical conditions where it is expected to degrade and dissipate faster (Carvalho et al. 1992) are little known. In a laboratory model aquarium simulating a tropical marine environment, we studied the persistence and accumulation of ^{14}C-chlorpyrifos in sediment, fish and oysters. The results obtained from these studies are reported in this paper.

MATERIALS AND METHODS

Ring-labelled ^{14}C-chlorpyrifos of specific activity 10.48 mC/mmol and 95% purity by TLC was obtained from the International Atomic Energy Agency

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(IAEA). Non-labelled chlorpyrifos standard was obtained from Greyhound Chromatography and Allied Chemicals, UK. Crystalline 2,5-diphenyl oxazole (PPO), 2,2-*p*-phenyl bis(4-methyl-5-phenyl oxazole) (dimethyl-POPOP) for liquid scintillation cocktail preparation were obtained from Fischer Chemicals, Fairlow, New Jersey, USA. Triton X-100 from John and Haas Co., USA was used for preparation of cocktail for radioassay of aqueous samples. Harvey Carbon ^{14}C-cocktail, for absorption of $^{14}CO_2$, was obtained from J.H. Harvey Inst. Corp, USA. Radioactivity was quantified using a Tri-Carb 1000 TR liquid scintillation counter. Combustion of solid radioactive samples for the $^{14}CO_2$ assay was performed in an OX-600 Harvey Biological Oxidizer. Metabolites were confirmed in a GC-MS, a GC HP 5890 Series II and MS Finnigan SSQ 7000 model; column: J&W DB-5ms, 60 m, i.d. 0.25 mm, film thickness 0.10 µm. The temperature program was: 60 °C (1.5 min); 7.5 °C/min to 260 °C (12 min), injection temperature: 250 °C, 1 µL splitless injection: carrier gas: helium; pressure: 25 psi. MS conditions: full scan from mass 50 to 500, cycle time 0.7 sec, temperature: 150 °C.

A model tropical marine ecosystem was prepared in the laboratory using a glass tank (68 cm x 36 cm x 36 cm) containing 52 L of seawater (pH 8.14) and 9.5 kg of wet sediment (composition: 88.5% sand, 11.7% silt, 0% clay, <0.06% carbon) taken from Gazi, North Coast at Mombasa along the Indian Ocean coast. The seawater was stored in the dark for two weeks to reduce algal growth. Algal growth was previously found to be too rapid in the aquarium under prevailing laboratory conditions. The aquarium was fitted with a thermostat to keep the temperature constant at 24 °C and mild pump aeration was provided. 30 oysters (*Isomonas alatus)* (attached to a wooden rack) and 25 each of the fish speces, *Luthrimus fulviflama* (snapper fish) and *Seganus stellatus* (rabbit fish) were added to the aquarium and the aquarium was left to stabilize for six weeks before starting the experiments.

In an experiment conducted to study the uptake of ^{14}C-chlorpyrifos in oysters, fish and sediment, the aquarium seawater was dosed with a mixture of ^{14}C-labelled and non-labelled standard of the compound to a concentration of 8.2 ppb every 24 hours for 7 days to keep the concentration in water constant and samples were taken (in duplicate) for analysis of ^{14}C-residues at various time intervals. In another experiment conducted to determine the depuration of ^{14}C-chlorpyrifos from oysters, fish and sediment into fresh seawater, the aquarium seawater was dosed to a concentration of 5.2 ppb every 24 hours for 7 days. In this experiment, the depuration of residues was investigated by analysing ^{14}C-residues released in the water and those left in the sediment, fish and oyster samples, respectively, taken at various intervals of time, replacing all the dosed seawater every 24 hours for 7 days.

The dissipation and distribution of ^{14}C-chlorpyrifos in a seawater/sediment system under static conditions, after dosing with ^{14}C-chlorpyrifos to a concentration of 48 ppb, as well as the rate of volatilization of ^{14}C-chlorpyrifos from a seawater-only

system (initial seawater concentration of 26.6 ppb) were also determined in separate experiments.

Residue analyses for chlorpyrifos concentrations in fish and oyster tissues, sediment and seawater were conducted separately. Fish samples (about 2.5 g each) were cut into small pieces with a knife, mixed with Na_2SO_4 in a pestle and mortar and ground to a fine mixture before extracting the residues in a Soxhlet apparatus with 120 ml methanol for 2.5 hours. The extracts were concentrated to 10 ml and aliquots taken for analysis by liquid scintillation counting (LSC). The remaining solid samples were combusted in the Oxidizer to determine the bound ^{14}C-residues. Sediment samples were analysed by combusting 1.5 g dried samples in the Oxidizer and analysing the $^{14}CO_2$ produced. Dry samples (50 g) of sediment for GC analysis of residues were extracted by Soxhlet with 120 ml CH_2Cl_2 for 4 hours. Water samples were analysed by taking 1 ml samples into 6 ml cocktail for LSC. The counting cocktail for water samples was made by mixing 4 g PPO and 0.1 g dimethyl-POPOP in 1 litre of 33% triton X-100 in toluene and that for organic samples by mixing 4 g PPO and 0.25 g dimethyl-POPOP in 1 litre of toluene. Other water samples (10 ml) were shaken in a separatory funnel with 30 ml dichloromethane three times, the extracts were combined and concentrated to small 5 ml samples in a rotary evaporator after drying with Na_2SO_4 for GC-MS.

RESULTS AND DISCUSSION

The dpm values obtained from the LSC analysis were converted to ng and expressed as ng/g (ppb) or ng/mL (ppb) of sediment/biota and water, respectively. The recovered ^{14}C-chlorpyrifos residues include the parent chlorpyrifos and any ^{14}C-chlorpyrifos metabolites. However, analysis of metabolite residues in sediment and water samples, taken at various intervals, by GC-MS over 28 days of exposure showed that chlorpyrifos had not degraded to any detectable metabolites in seawater and sediment during the experimental period. The GC-MS spectra showed <5% change of chlorpyrifos in seawater and sediment over this period. The results of the studies are summarized in the following Figure1 and Tables 1, 2 and 3.

In our studies, only chlorpyrifos was detected in the seawater and sediment extracts by GC-MS. This was unexpected because transformation of chlorpyrifos in various environmental compartments have been reported in previous studies. It is possible that our compound quickly partitioned into sediment and biota and therefore had very little time to hydrolyse to any significant extent in the seawater over the 28-day experiment period. Alkaline hydrolysis of chlorpyrifos to 3,5,6-trichloro-2-pyridinol (TCP) has been reported in freshwaters and is considered to be one of the dominant removal processes of this toxic compound there (Liu et al. 2001). Another mechanism of hydrolysis of chlorpyrifos occuring by neutral hydrolysis to desethyl chlorpyrifos [O-ethyl-O-hydrogen-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] has also been reported (Liu et al. 2001; Meikle et al. 1978). The hydrolysis of chlorpyrifos in water is known to be pH and temperature

dependent and its resistance to hydrolysis in water is higher in acidic conditions (Sharom et al 1980; Meikle et al. 1978).

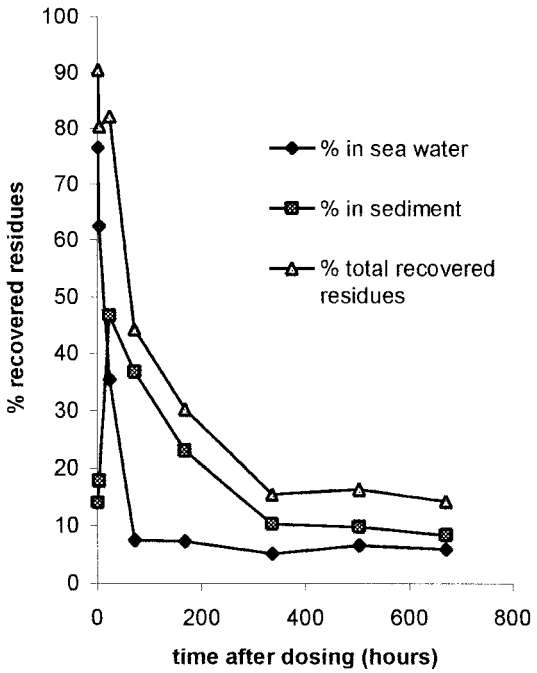

Figure. 1. Distribution of ^{14}C-chlorpyrifos residues (expressed as % of initial applied amount) recovered in seawater and sediment .

Degradation of chlorpyrifos in seawater-flooded sediment has also been reported by Kale et al (1997) who found 74.9% conversion to TCP after 40 days exposure and in sediment, in a laboratory marine ecosystem, by Carvalho et al (1992). Very short hydrolysis half-lives of chlorpyrifos have been reported in river waters (24-126 days, pH 7.93-7.99), in pond waters (1.41 days, initial dose of 0.10 ppm) and canal waters (1.54 days, pH 8, initial dose of 0.10 ppm) (Meikle et al 1978; Liu et al 2001; Sharom et al 1980). Table 1 shows that chlorpyrifos volatilized very rapidly from seawater leaving only 4.5% of the applied dose in the water after 28 days. We obtained a half-life of dissipation from seawater of 5.9 days by first-order kinetic analysis of our data. In the seawater/sediment ecosystem, the half-life of dissipation from seawater was 7.8 days and an overall half-life of dissipation from the combined seawater/sediment compartment was 10.3 days. Chlorpyrifos was rapidly deposited onto sediment within the first 24 hours after dosing and we obtained a peak concentration of 20 ppb in sediment after 24 hours. However, after 28 days, only 5.9% (2.5 ppb) and 8.4% (3.6 ppb) of the

applied pesticide were detected in seawater and sediment, respectively, as shown in Figure 1.

Table 1. The dissipation of ^{14}C-chlorpyrifos from seawater at 24 °C.

Time (hrs)	concentration of ^{14}C-chlorpyrifos in water ppb	remaining residues as % age of initial applied amount (%).
0	26.6	100
2	24.6	92.5
4	17.9	67.3
24	14.7	55.3
168	10.6	39.8
336	1.2	4.5
504	1.2	4.5
672	1.2	4.5

Note: The compound was dosed once.

Our results were comparable to those obtained by Carvalho et al (1992). Carvalho et al reported adsorption to fine sediment as a reason for increased persistence of chlorpyrifos in temperate seawater/sediment ecosystems. After only 2 days of exposure they found 15% of applied chlorpyrifos in the sediment.

Table 2. Distribution of ^{14}C-chlorpyrifos residues in sediment, water, oysters and fish (ppb).

Time (hrs)	Water (ppb)	Sediment (ppb)	Oysters (ppb)	*Fish (LLF) (ppb)	**Fish (LFC) (ppb)
2	1.6	5.1	334	330	526
		(3.3)	(216)	(216)	(339)
4	1.5	3.5	208	349	690
		(2.4)	(142)	(238)	(469)
8	2.4	5.9	217	274	508
		(2.4)	(89)	(112)	(208)
24	1.5	5.0	278	235	372
		(3.3)	(183)	(155)	(245)
48	2.1	6.8	483	367	529
		(3.2)	(226)	(171)	(247)
72	2.4	8.9	315	390	637
		(3.7)	(131)	(163)	(265)
120	3.7	5.5	164	346	201
		(1.5)	(45)	(95)	(55)
168	4.1	5.8	450	421	584
		(1.4)	(111)	(103)	(144)

Note: the values in parenthesis in columns 3, 4, 5 and 6 represent bioaccumulation factors (BAF_w) based on the determined water concentrations; *LLF: *Luthrinus fulviflama* (Snapper fish); **LFC: *Siganus stellatus* (Rabbit

fish). The animals were exposed statically but the concentration level of the pesticide in the aquarium water was kept fairly constant by additional dosage every 24 hours until the end of the experiment.

We observed rapid uptake of ^{14}C-chlorpyrifos in oysters and fish and obtained the highest bioaccumulation factors (BAF_w) of 469 (rabbit fish, after 4 hours), 238 (snapper fish, after 4 hours) and 226 (oysters, after 48 hours). The BAF_w values were calculated by dividing the tissue concentrations (ppb) by the water concentrations (ppb) at the times of sampling. The bioaccumulation was found to be very rapid initially but, due to excretion, the BAF_w values were lower especially for samples taken after 120 hours of exposure. As shown in Table 2, whole tissue concentrations were much higher than some of the reported toxic concentrations even though we did not determine metabolism in these organisms. However, high concentrations in tissues can pose dangers to predators. Rapid uptake of chlorpyrifos in different fish and mollusc species has also been reported with bioconcentration factors (BCF) in the range of 150-650 (Carvalho et al 1992).

Table 3. Depuration of ^{14}C-chlorpyrifos from sediment, oysters and fish into clean seawater expressed as detected amounts in ppb.

Time (hrs)	water (ppb)	sediment (ppb)	oysters (ppb)	fish (LLF) (ppb)	fish (LFC) (ppb)
2	0.77	4.45	184.9	503.7	181.2
4	1.10	7.70	178.1	493.0	193.5
8	0.83	3.65	176.3	381.3	189.3
24	1.13	3.06	140.1	320.4	171.4
48	1.01	14.58	144.7	267.8	170.3
72	1.21	3.84	143.5	300.3	145.5
120	0.80	3.01	127.6	250.9	150.4
168	0.93	0.48	119.0	195.0	110.3

As shown in Table 3, we found the excretion to be rather slow and high concentrations of ^{14}C-residues were still recoverable after 7 days in oysters (64% of accumulated residues), snapper fish (32.3%) and rabbit fish (60.9%). However, excretion from oysters and rabbit fish particularly seemed to be more rapid within the first 2 hours of exposure to clean seawater. By first-order kinetic analysis of data obtained between 2 – 168 hours, excretion half-lives of 5.6 days and 10.0 days were obtained for snapper fish (LLF) and rabbit fish (LFC), respectively, after 7 days continuous exposure to clean seawater. The elimination rate was lower in oysters with an excretion half-life of 12 days. The recovered residues could include both the parent compound and its degradation metabolites. More rapid excretions have been reported for other organophosphorous compounds. Mansingh and Robinson (1997) have reported 42% (in 1 hour) and 47% (in 4 hours) excretion of isazofos and diazinon in red hybrid tilapia, respectively, followed by complete elimination after 72 hours continuous exposure to clean seawater.

Our laboratory investigations showed that chlorpyrifos has a very short lifetime in seawater ($t_{1/2}$ of 7.8 days) in a seawater/sediment system where it partitions very rapidly into sediment. This rapid distribution into sediment can extend its residual lifetime although concentration levels in real field samples of seawater and sediment are not yet known. Like other organophosphates, its persistence in the aquatic environment is expected to be low, particularly, under tropical conditions where high solar radiation intensity and elevated temperatures can accelerate its volatilization and degradation rates. The results of our experiments also showed that chlorpyrifos is capable of bioaccumulating rapidly in oysters and fish in contaminated seawater and therefore bears potential toxicity to these organisms and their predators. Its excretion rates (based on whole tissue concentrations after continuous exposure to clean seawater) from oysters and fish were also relatively slow, as indicated by the data in Table 3. However, in fish, mammals and birds, detoxification of this toxic compound is known to occur by hydrolysis to 3,5,6-trichloro-2-pyridinol and by dealkylation to give trichloropyridyl phosphate (Ongeri 1998, Smith et al 1967).

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