Appendix

Publication and presentation.

1. Abdullah, A.R. and Shanmugam, S.S. (1995) Distribution of lindane in a model mudflat ecosystem. *Fresenius Environ. Bull.*, 4: 497-502

2. Shanmugam S.S, Abdullah A.R., Juzu, H.A. and Wong, K.Y (1996) Fate and Distribution of [^{14}C]Lindane and [^{14}C]DDT in a Model Mudflat Ecosystem. This paper was presented at the third International Atomic Energy Agency (IAEA) RCM meeting, 9-13 September 1996, Costa Rica.

Fresenius Envir Bull 4: 497-502 (1995)
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1018-4619/95/080497-06 $ 1.50+0.20/0

DISTRIBUTION OF LINDANE IN A MODEL MUDFLAT ECOSYSTEM

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Summary: The distribution of lindane was investigated in a model mudflat ecosystem comprising of estuarine water, sediment and cockles (*Anadara granosa*). When exposed to 8 and 15 µg/L of the insecticide under semistatic conditions, for a period of 30 days, a gradual increase in lindane concentration was observed in both sediment and cockles, while the concentration of lindane in water ranged between < 0.01-0.73 µg/L at the end of 24h exposures. The half life of lindane in estuarine water was found to be 21.9h under aerated conditions and 43.0h without aeration. Microbial degradation is implicated in the persistence of lindane in estuarine water.

Key words: Lindane, mudflat, estuarine water, sediment, cockles (*Anadara granosa*)

INTRODUCTION

In Malaysia, hexachlorocyclohexane (HCH) has been used for decades for agricultural and horticultural purposes, and is one of the few remaining organochlorine (OC) insecticides still being widely used. Technical HCH is a mixture of five isomers, designated as α, β, γ, δ and ε. Lindane is the common name for the γ-isomer of HCH.

The rate of degradation of lindane in the environment is slow. It has a half life in the field exceeding 100 days, subjected to factors such as the characteristics of the soil, location and climate (1). Its persistence in the aquatic environment is influenced, amongst other factors, by the physico-chemical properties of the water body (2). In freshwater, a half life of 65 hours has been recorded for lindane (3). In Malaysia, several surveys over the years have shown the presence of HCH, including lindane, in both biotic and abiotic components of the freshwater (4-6), as well as the marine environment (7).

The mudflats along the western coast of Peninsular Malaysia are commonly used for the culture of the economically important marine bivalve, *Anadara granosa*. Some of the major agricultural areas are also located in coastal plains which are in the vicinity of the culture beds. Hence, applied pesticides, including lindane may enter the mudflats as a result of spraydrift and runoff from agricultured land. Information on the fate and distribution of pesticides in the mudflats is of considerable importance not only with respect to possible ecological impacts but also in the interest of public health when shellfish from this area are available for consumption.

The present paper examines the distribution of lindane in a model mudflat ecosystem comprising of estuarine water, sediment and cockles (*Anadara granosa*) exposed to low concentrations of the insecticide under semistatic conditions, for a period of 30 days. In a related experiment, the persistence of lindane in estuarine water was also determined.

MATERIALS AND METHODS

Estuarine water, sediment and cockles (*Anadara granosa*) used in this study were collected from the mudflats of Kuala Selangor, Peninsular Malaysia, and maintained in fibre-glass tanks for a period of 40 days prior to the commencement of the experiments. Water, sediment and cockles were also analysed for the possible presence of lindane. None was detected.

The sediment used in this study was analysed to be mainly a mixture of sand, silt and clay with the following particle size distribution, expressed as % of total dry weight: 2.0 mm (0.1%), 1.0-2.0 mm (0.4%), 0.5-1.0 mm (1.1%), 0.25-0.5 mm (16.7%), 0.125-0.25 mm (15.3%), 0.063-0.125 mm (30.9%), 0.063 mm (35.5%). Organic content was determined as loss on ignition of 20 g of dried sediment for three hours at 550 °C. A mean value of 8.9% of dry weight was obtained.

The model ecosystems were established in 50L glass tanks with aeration (dissolved oxygen concentration averaged 6.8 mg/L). Some physico-chemical properties of the estuarine water were as follows (mean values): temperature, 24.4 °C; alkalinity, 110 mg $CaCO_3$/L; pH, 8.6 and salinity, 32.5 ppt.

Appropriate quantities of the stock solution of analytical-grade lindane (99.0 % purity, Chem Service Inc. USA) in pesticide-grade acetone were added to 9L of estuarine water and stirred for 15 min, to achieve lindane concentrations of 5 and 15 µg/L. The model ecosystems were prepared by carefully pouring the above solutions into the tanks containing sediment (typically about 3 kg), within which the cockles (50 in number, biomass about 30g) were embedded. The experiments were carried out under semistatic conditions whereby the solutions were renewed every 24h. Sediment and cockles were randomly sampled at specific time intervals and kept at -20 °C prior to analysis. Water samples (500ml) were analysed just prior to pouring into the tanks and 24h later before renewal.

The experiments to determine the persistence of lindane in estuarine water were conducted using identical conditions to the model ecosystem but without sediments or cockles. The experiments were conducted in both aerated and non-aerated conditions under natural light. Aliquots (500ml) were removed daily for 4 days and analysed for lindane. This experiment was conducted inconjunction with distilled water, in place of estuarine water.

Water samples (500 ml) were extracted three times with hexane (150ml). After drying with sodium sulfate, the solvent was evaporated to dryness, the residue redissolved in 5ml hexane and injected directly into the gas chromatograph. Extractions carried out on spiked samples (15 µg lindane in 1 L water) gave recoveries averaging 93% while the standard deviations of the method employed was less than 6%. Thawed sediment samples were extracted after the excess water was removed by suction filtration followed by freeze drying. Dried sediment was soxhlet-extracted in triplicate with hexane for 8h followed by dichloromethane for another 8h. The dichloromethane

was evaporated to dryness, the residue redissolved with the hexane phase, and then concentrated to about 5ml. Sulphur was removed by treatment with mercury, and finally the extract concentrated for column chromatography. Pooled cockles (4-7 individuals) were deshelled, excess water removed and the tissues cut into small pieces and freeze-dried. Dried tissue was soxhlet-extracted in triplicate, with hexane for 8h, and then concentrated. Lipids were removed by treatment with concentrated sulphuric acid, and finally the extract was concentrated for column chromatography.

Florisil column chromatography (5% deactivated with water) was used for the clean-up by elution with solvents of increasing polarity (70 ml hexane followed by 50 ml hexane/dichloromethane (70:30) and finally 40 ml dichloromethane).

A Shimadzu GC 17A gas chromatograph interfaced to a QP-5000 mass spectrometer was used for the quantitative analysis of lindane. A HiCap OV 1701 fused silica capillary column (25m x 0.2 mm i.d.) was programmed from 60°C to 100°C at 5°C/min, held at 100°C for 10 min, and from 100°C to 230°C at 8°C/min. The ion source and analyzer were maintained at 250°C. Helium was used as the carrier gas at a flow rate of 40 cm^3/min. Injection volume was 2 µL and the splitless mode was used. The ionization potential was 70 eV. Lindane was quantified using selected ion monitoring (m/z: 111, 181, 183 and 219). Calibration was carried out using external standards. The mean recovery rates of lindane from sediment and cockles were 93% and 85% respectively. Using samples spiked with 20 µg lindane, standard deviations of the methods employed was found to be less than 11% for sediment samples and less than 15% for tissue samples.

RESULTS AND DISCUSSION

The persistence of lindane in estuarine water was reduced substantially by aeration (Figure 1).

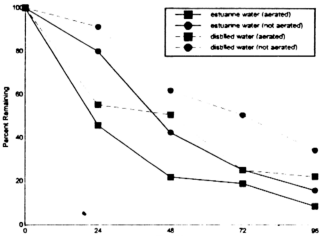

Figure 1: Persistance of lindane in estuarine and distilled water under aerated and non-aerated conditions. Each point represents a mean of triplicate determinations. The percent recovery was calculated relative to the initial amount measured and corrected to 100 %.

The half lives of lindane under aerated and non-aerated conditions were 21.9 and 43.0h respectively. Lindane under aerated conditions showed a dissipation of 54.3% at the end of 24h and a loss of 91.5% at the end of the experiment (96h). When not aerated, only 20% loss was observed at the end of 24h, while at the end of 96h, 84.3% had dissipated. Lindane has a vapour pressure of 9.4×10^{-6} mm Hg at 25°C (8) and is more volatile than most OC insecticides. This, coupled with the high atmospheric temperature (typically 28-31°C), and accelerated by aeration resulted in the observed rapid rate of dissipation. The half live of lindane was also found to be greater in distilled water than in estuarine water (Figure 1). The half lives of lindane in distilled water under aerated and non-aerated conditions were 49.4 and 73.4h respectively. The greater rate of degradation in estuarine water is suggestive of biological degradation. In previous studies, both microbial action and alkalinity has been implicated in the degradation of lindane (9).

Table 1 shows the levels of lindane detected in water, sediment and cockles at different time intervals at exposure concentrations of 5 µg/L and 15 µg/L. Water in the model ecosystem was analysed upon preparation of the solution and again immediately before renewal of the solution. Measured initial concentrations were not less than 95% of the nominal concentration. At the end of 24h, prior to renewal of the solutions, the concentration of lindane in water ranged between <0.01-0.24 µg/L at a exposure concentration of 5 µg/L and <0.01-0.73 µg/L at a exposure concentration of 15 µg/L. The rapid disappearance of lindane over a 24h period is attributed mainly to volatilization, in addition to sorption into sediment and bioconcentration in the cockles. A small amount of sorption to the glass surface of the tanks may also have occurred (10).

Table 1: Concentration of lindane in water, sediment and cockles exposed to 5 and 15 µg/L of the insecticide under semistatic conditions.

Concentration of lindane[a]	Exposure Period (days)							
	4	6	8	11	15	19	25	30
Exposure concentration: 5µg/L								
Water[b] (µg/L)	<0.01	<0.01	0.24	<0.01	0.02	<0.01	0.15	0.01
Sediment[c] (µg/kg)	4.39	9.24	12.99	15.45	16.46	22.90	30.06	46.12
Cockles[c] (µg/kg)	8.07	8.76	12.60	16.37	24.02	25.77	33.59	33.05
Exposure concentration: 15µg/L								
Water[b] (µg/L)	<0.01	<0.01	0.08	0.12	0.06	0.73	0.30	0.50
Sediment[c] (µg/kg)	19.19	26.50	30.67	32.40	32.60	45.80	51.70	82.19
Cockles[c] (µg/kg)	13.32	16.50	22.13	25.79	43.30	44.72	50.68	56.19

[a] mean value of triplicate determinations
[b] concentration at the end of 24h
[c] dry weight basis

The concentrations of lindane in both sediments and cockles increased throughout the duration of the experiment. At the end of the experiment the concentrations of the insecticide in sediment and cockles were found to be 46.1 and 33.1 µg/kg respectively at a exposure concentration of 5 µg/L. When exposed to 15 µg/L lindane, the concentrations of the insecticide in sediment and

cockles at the end of the experiment were 82.2 and 56.2 μg/kg respectively. Although these results, particularly the latter result indicated that the concentration of lindane in sediment was significantly higher than in cockles, this trend was not significant nor consistent for samples taken at other times.

As is characteristic of organochlorine insecticides, the presence of lindane in sediment was more significant than in water. The sediments used in this study are very rich in organic matter (8.9% on a dry weight basis), which act as a reservoir for the OC insecticide. Previous studies have shown that the organic matter content, in the form of humic substances, play a major role in the accumulation of chlorinated hydrocarbons in sediments (11).

The ability of aquatic organisms to concentrate organochlorine compounds many times above levels found in water has been amply demostrated and documented. In studies involving bioaccumulation of lindane in bivalves, bioaccumulation factors ranging from 110 in the mussel *Mytilus edulis* (12) to 2610 for the freshwater clam *Corbicula manilensis* (13) have been reported. In the present study, with the semistatic nature of the exposure, the concentration of lindane in the cockles increased with increasing time. As no steady state was reached during this period, bioconcentration factors could not be estimated as the ratio of the concentration of the insecticide in cockles and water at equilibrium. However, it is noteworthy that no mortalities were observed throughout the duration of the study, thus providing evidence of its resistance to stress and high accumulation of the chemical.

Results obtained in the present study are consistent to a similar study conducted with a model aquatic ecosystem, where volatilization accounted for more than 50% of the loss of the applied lindane (10). Furthermore, a flux model for the watershed of the Vellar estuary in South India, which has very similar climatic conditions to those in the present study also indicated that most of the HCH applied is removed by their volatilization into the atmosphere (14).

The results of this study clearly demonstrated the roles of various processes in determining the fate of lindane in the model mudflat ecosystem. In addition to volatilization, which appeared to be a major pathway resulting in the depletion of the insecticide in the water column, sorption to sediments, as well as bioconcentration by cockles were observed to be increasingly significant with continued exposure. Bioconcentration of lindane in cockles might present some degree of risk to human health from the consumption of the bivalve, particularly taking into consideration that cockles are normally marketed immediately after harvest without depuration. Furthermore, the presence of lindane within cockles and sediments also serve as an indication for the extent of pollution due to the insecticide.

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Accepted 30 March 1995

Fate and Distribution of [^{14}C]Lindane and [^{14}C]DDT in a Model Mudflat Ecosystem.

(Agency Research Contract No. 7938/MC)

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Third FAO/IAEA RCM, Costa Rica, 9-13 September, 1996.

Volatilization of [^{14}C]-γ-HCH from a Model Mudflat Ecosystem.

Introduction

Volatile pesticides are more rapidly lost in the tropical agroecosystem due to the high temperatures associated with this region, where maximum temperatures may be as high as 34^{0}C. Pesticides such as HCH which have a vapor pressure of 9.4 x 10^{-6} mmHg have been shown to undergo rapid volatilization from tropical agroecosystems (Tanabe *et al.*, 1992; Ramesh *et al.*,1989), as well as other environments (Takeoka *et al.*, 1991 ; Abdullah and Shanmugam, 1995). In excess of 90% of the HCH applied to a paddy field in India dissipated within two weeks by atmospheric transport (Tanabe *et al.*, 1992).

Previous investigations with the model mudflat ecosystem revealed the importance of volatilization as a route of dissipation for γ-HCH (Abdullah and Shanmugam, 1995). It is the aim of this study to further elucidate the process of volatilization in a model mudflat ecosystem consisting of soil and water taken from the mudflats of Kuala Selangor.

Materials and Methods

A diagram of the manifold assembly system used to study the volatilization based on the method described by Kearney and Kontson (1975) is shown in Figure 1. It consisted of five Fernbach flasks and a 5L round bottom flask containing the model ecosystem ; two flasks contain 0.5M KOH to remove CO_2 from the incoming air and another contains water to maintain a saturated moist atmosphere in the model ecosystem. Thereafter was placed a Fernbach flask, containing 2-ethoxyethanol to absorb volatile compounds. This was then connected to a NaOH (0.25M) trap to absorb released $^{14}CO_2$. Volatile organic compounds including parent HCH, as well as any degradation products would be trapped by the polyurethane plug placed in the model ecosystem as well as in the 2-ethoxyethanol. The air flow was maintained at about 5ml min^{-1}. The mudflat soil used in this experiment was mainly a mixture of sand, silt and clay with the following particle size distribution, expressed as percentage(%) of total dry weight : 2.0mm(0.1%), 1.0-2.0mm(0.4%), 0.5-1.0mm(1.1%), 0.25-0.5mm(16.7%), 0.125-0.25mm(15.3%), 0.063-0.125mm(30.9%), 0.063mm(35.5%). Organic content was determined as loss on ignition of 20g of freeze-dried sediment for three hours at 550^{0}C. A mean value 8.9% of dry weight was obtained.

The model ecosystem consisted of soil (1000g) and estuarine water (1litre) taken from the mudflat in Kuala Selangor, allowed to acclimatized in the manifold assembly for a period of 2 weeks after which was added [^{14}C]-γ-HCH (sp. act. 27.40 mCi/mmol obtained from IAEA, Monaco) prepared in ethanol to a final

concentration of 1.5mgL^{-1}. A control experiment was also conducted without the insecticide.

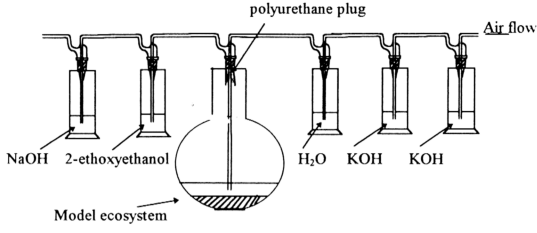

Figure 1. Diagram of the manifold system used to measure volatilization of γ-HCH in mudflat ecosystem

Data on the formation of volatile organic compounds and $^{14}CO_2$ evolution were obtained at the end of 7 days, 14 days and 21 days. At the end of the appropriate incubation periods, the soil was filtered. The filtered soil was extracted with acetone (250ml) followed hexane (250ml) in a Soxhlet apparatus. The resulting extractable residues were analyzed by LS counting the corresponding bound material was determined by combustion in a Biological Oxidizer (R.J Harvey OX 600). Water samples were extracted with hexane (50mlx2) and the radioactivity was directly measured by LS counting

A 0.5ml aliquot from NaOH trap plus 10ml of scintillation cocktail (PPO, 5.5g ; POPOP, 0.1g ; toluene, 667ml ;triton X-100, 330ml) and 0.5ml from 2-ethoxyethanol trap plus 10ml of scintillation cocktail were analyzed by liquid scintillation (LS) counting (Beckmen LS 6000 SE). The polyurethane plugs were extracted in a Soxhlet apparatus with hexane (150ml) and ^{14}C activity determined by LS counting. All samples were corrected for background and quenching.

Results and Discussions

The distribution of ^{14}C activity in the various components of the manifold assembly expressed as percentage of the applied activity is shown in Table 1.

% of Applied Activity							
Time (days)	$^{14}CO_2$	Volatile Compounds		Water	Soil		Total
		2-Ethoxyethanol	Polyurethane plug		Extractable	Bound	
7	0.02 (0.04)*	0.1 (0.2)	6.64 (11.5)	0.05 (0.09)	48.06 (83.1)	2.99 (5.2)	57.86
14	0.12 (0.2)	0.14 (0.2)	6.57 (10.5)	0.03 (0.05)	51.63 (82.4)	4.23 (6.75)	62.72
21	0.2 (0.4)	0.16 (0.2)	13.46 (16.8)	0.03 (0.04)	61.42 (76.7)	4.95 (6.17)	80.22

*Figures in parentheses indicate the actual value corrected to 100%.

Table 1. Distribution of ^{14}C recovered from [^{14}C]-γ-HCH treated model ecosystem

The maximum organic volatile was observed at the 21-day sampling period. LS counting of the hexane extracts of the polyurethane plugs showed that, it is able to trap the volatile labeled material more than the 2-etoxyethanol. Polyurethane plugs was reported to be very efficient in trapping the volatile organic compounds in a previous study (Kearney and kontson, 1975).

TLC analyses of the hexane extracts of the polyurethane plugs and 2-etoxyethanol traps showed that all the radioactivity that was spotted had disappeared from the plate. Thus, it appears that the compounds trapped in polyurethane plugs and 2-ethoxyethanol were extremely volatile. GCMS analysis of hexane extracts and 2-ethoxyethanol also did not show the presence of γ-HCH. These observations are consistent to a previous study conducted by Drego *et al.* (1990).

Maximum production of $^{14}CO_2$ was 0.2% of the applied activity at the end of the 21 days incubation period. The level of $^{14}CO_2$ in these experiments were significantly lower compared to those reported in previous studies. In an investigation using labeled γ-HCH in paddy ecosystem, Drego *et al.*, (1990) reported that a total of 12.3 and 10.7% of the applied radioactivity was found to be evolved as $^{14}CO_2$ in green manure-amended and unamended soil, respectively. Brahmaprakash *et al.*, (1985) also reported, the evolution of CO_2 from flooded soils planted and unplanted with rice amounted 1-2% of the originally applied ^{14}C

at 30 days. Kohnen *et al.*, (1975) reported that 6% of the applied ^{14}C activity of γ-HCH was evolved as $^{14}CO_2$ after 71 days and 17.8% as $^{14}CO_2$ after 140 days from submerged soil. Scheunert *et al.*, (1987) reported 3% of the applied [^{14}C]-γ-HCH was mineralized to $^{14}CO_2$ at the end of 42 days.

The radioactivity levels in the water were 0.05, 0.03 and 0.03 % at the end of 7 days, 14 days and 21 days respectively. These low levels were consistent with the earlier experiment with non-labeled γ-HCH where less than 0.05% were recovered after 4 days (Abdullah and Shanmugam,1994). Residue levels of γ-HCH in the water phase of paddy- field ecosystem have also revealed relatively low levels of parent compound soon after introduction of the insecticide (Drego *et al.*, 1990; Scheunert *et al.*, 1987).

The extractable radioactivity from the soil were 48.06, 51.63 and 61.42 % at the end of 7 days , 14 days and 21 days, respectively (Table 1). The extractable radioactivity level are higher than those reported earlier by Drego *et al.*, (1990). However in the present experiment, it shows that most of the radioactivity remained in the soil probably due to the high organic matter content of the soil. TLC plate and GCMS analysis also confirmed the presence of γ-HCH in the soil extract. Soil bound residue of γ-HCH constituted 2.99, 4.23 and 4.95% of the applied radioactivity at the end of 7 days, 14 days and 21 days, respectively. The formation of bound residues from ^{14}C-γ-HCH was also noted to increase with time.

The ratio of pesticide volatilized to pesticide metabolized were 332, 56 and 68 for the period of 7-day, 14-day and 21-day, respectively. The amount lost as volatile organic materials tended to decrease significantly from 7 days to 14 days and 21 days. From these observations it is evident that γ-HCH tend to volatilized soon after introduction of the insecticide. However, the ratio of pesticide volatilized to pesticide metabolized will depend on many factors such as soil properties, moisture, air exchange rate and others.

From Table 1, it can be seen that 57.86, 62.72 and 80.22% of the applied activity could be accounted for at the end of 7 days, 14 days and 21 days, respectively. The unaccounted radioactivity could have been lost during the process of soil filtration. The results obtained from this experiment indicate that volatilization is one of the important pathways involved in the dissipation of γ-HCH from the mudflat environment. Futhermore the observation of $^{14}CO_2$ was taken as indication of metabolism, arising from microbial degradation in the soil.

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Adsorption of γ-HCH in Several Malaysian Soils.

Introduction

Adsorption of pesticides in soils is an important factor affecting the fate of pesticides in the soil environment. The extent of adsorption depends primarily on the chemical nature of the pesticide and soil properties. For non-polar organic compounds the role of organic matter in the soil has been shown to be significant (Lichtenstein and Schulz, 1959 ; Bialey and White, 1964 ; Edward, 1966 ; Nash and Woolson, 1967 Sanchez-Martin and Sanchez-Camazano, 1991; Arienzo *et al.*, 1994).

A preliminary investigation with the model mudflat ecosystem revealed the importance of adsorption as a route of dissipation for γ-HCH (Abdullah and Shanmugam, 1995). In this study the adsorption of γ-HCH in the mudflats and two agricultural soils were examined.

Materials and Methods.

The pH and organic matter content of the soils studied is shown in Table 1.

Sample	pH	Organic Matter (%)
Mudflat soil (MS)	5.05	8.9
Agricultural soil (A)	4.45	7.6
Agricultural soil (B)	4.70	8.4

Table 1. pH and organic matter content of the soils studied.

Soil MS was collected from the mudflats in Kuala Selangor while soil A and soil B were from agricultural areas. Samples were air dried and sieved through 2.5mm mesh. Organic matter content was determined as loss on ignition of 20g of air-dried soil for three hours at 550^0C.

The γ-HCH adsorption isotherms for the different soils were obtained by treating triplicate samples of 20g of soil with 20ml of an aqueous solution of the γ-HCH at a concentration of 2.4, 3.4, 4.8, 7.3 and 9.8 μg/ml. Formulated lindane (active ingredient: γ-HCH: 20.3%; inert material: 79.7%. supplied by Wesco Agencies, Malaysia) used was prepared in water. The suspensions were kept at 30^0C for 24 hours in a thermostat-controlled chamber with circulatory shaking motion. Samples of 20g soil with 20ml deionized water were also maintained as blanks.

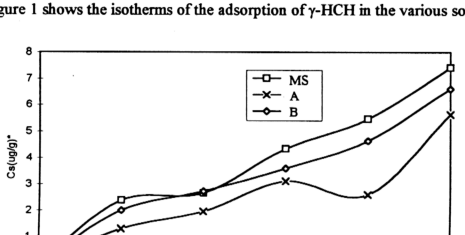
At the end of 24 hours the soil suspensions were centrifuged and the supernants were extracted with hexane (5mlx2) , and finally the extract was concentrated for column chromatography.

Florisil column chromatography (5% deactivated with distilled water) was used for the clean-up by elution with solvents of increasing polarity (70ml of AR grade hexane, 50ml of AR grade hexane/ dichloromethane (70:30) and finally 40ml of AR grade dichloromethane). The combined elutes were collected and then concentrated using rotary evaporator and the final volume adjusted to 5ml with GC grade hexane. This extract was directly analyzed by GCMS.

A shimadzu GC 17A gas chromatography interfaced to a QP-5000 mass spectrometer was used for the quantitative analysis of the γ-HCH. A HiCap OV 1701 fused silica capillary column (25m x 0.2mm i.d) was programmed from 60°C to 100 °C at 5 °C /min, held at 100 °C for 10min, and from 100 °C to 230 °C at 8 °C /min. The ion source and analyzer were maintained at 250 °C. Helium was used as the carrier gas at a flow rate of 40cm^3/min. Injection volume was 2μL and the splitless mode was used. The ionization potential was 70eV. γ-HCH was quantified using selected ion monitoring (m/z: 111, 181, 183 and 219). Calibration was carried out using external standards. Extractions carried out on spiked samples (20 μg γ-HCH in 20 ml water) gave recoveries averaging 96% while the standard deviations of the method employed was less than 4 %.

Results and Discussion.

Figure 1 shows the isotherms of the adsorption of γ-HCH in the various soils.



* Mean of triplicate estimations.

Figure 1. Adsorption isotherms of γ-HCH on soils.

The adsorption isotherms of γ-HCH in soils with high organic matter (OM) content are of the L type (concave initial curvature) according to the classification of Giles *et al.* (1960), pointing to the great affinity of these soils for the pesticides. Soils with OM content lower than 2.4% results in isotherms of the S type (convex initial curvature) indicating low pesticide soil affinity at low pesticide concentration. The initial curvature concavity is higher in soils with a high OM content. The adsorption isotherms of all the soils examined in this study exhibited the L type, attributed to the high OM content of these soils.

The isotherms obtained generally conformed to the Freundlich adsorption equation with a correlation coefficient $r > 0.86$ This equation can be expressed in linear form as :

$$\log Cs = \log K + (1/n)\log Ce$$

Where Cs(μg/g) is the amount of adsorbed pesticide, Ce(μg/ml) is the equilibrium concentration of dissolved pesticide and K and n are two constants characteristic of the pesticide adsorption capacity. In fact, K is the amount of pesticide adsorbed at an equilibrium concentration of 1μg/ml, So its represents adsorption at low concentrations. On the other hand n reflects to what extent the pesticide adsorption is dependent on the concentration. The K and n values

obtained from isotherms, as well as K_d and Log K_{OM} values, are given in table 2. The distribution coefficient, K_d, represents adsorption at equilibrium concentration higher than K and is defined as the ratio between the pesticide concentration in the soil and that in the equilibrium solution at a given equilibrium concentration. (K_d was calculated for Ce = 5μg/ml, K_{OM} is the K value normalized to 100% OM, K_{OM} = 100 x K / %OM.)

Table 2. Constants and Correlation Coefficients of the Freundlich Adsorption equation (K, n and r), Distribution Coefficient (K_d) and logK_{OM}.

Soil	K	n	r	K_d[a]	LogK_{OM}
MS	1.40	1.05	0.97	1.40	1.20
A	0.76	0.99	0.97	0.83	1.00
B	1.25	1.03	0.86	1.21	1.17

[a] Ce = 5 μg/ml

Among the soils (MS, A and B), the largest K values (Table 2) corresponded to soil MS. K and K_d[a] values were very similar since n was essentially unity in all cases. LogK_{OM} values, which is frequently used as a measure of the pesticide adsorption capacity of soils were 1.20, 1.00 and 1.17 for soil MS, A and B, respectively. The order of adsorption was hence determined to be MS > B > A which was consistent with the organic matter content in the soils. The results obtained in this study indicated the role of OM content in the adsorption of γ-HCH by the soils.

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Biodegradation of γ-HCH in a Model Mudflat Ecosystem.

Introduction

Microbial degradation has long been recognized as a primary route of dissipation for many pesticides in soil and water ecosystems. The persistent nature of many chlorinated hydrocarbon insecticides have been attributed to the formation of a complex with some components of the environment which is largely resistant to microbial attack. Lindane has been identified among these recalcitrant molecules (Alexander, 1965).

However, lindane was first observed to be less persistent in submerged tropical soils by Raghu and MacRea (1966), who found only a small percentage of the applied insecticide remaining in the soil at the end of 90 days. The participation of microorganisms in the degradation of lindane was illustrated in the observation of the greater loss of the insecticide from non-sterilized flooded soil compared to sterilized soil under identical conditions. (MacRae *et al.*, 1969). The bacterium *Sphingomonas paucimobilis* isolated from the surface layer of HCH-treated flooded soil was observed to cause rapid degradation of the four isomers of HCH in the order $\alpha > \gamma > \delta > \beta$ under aerobic conditions (Bhuyan *et al.*, 1993).

In this study, the degradation of lindane in sterilized and non-sterilized soils taken from the mudflats was examined to assess the role of microbial degradation in the loss of lindane in the mudflats.

Materials and methods

Soil from the mudflat was collected from Kuala Selangor and maintained in a fibre-glass tanks with aeration for a period of one and a half months prior to the commencement of the experiment. To evaluate the significance of biodegradation of lindane in mudflat soil , a comparison of the persistence of lindane in sterilized soils and in nonsterilized soils was made. The soil was sieved (2.5mm. diameter) and sterilized by heating at 110 ^{0}C for 24 hours. The sample bottles (500ml) containing sterilized and nonsterilized soils were treated with formulated lindane (active ingredient: γ-HCH: 20.3%; inert material: 79.7%. supplied by Wesco Agencies, Malaysia) at the rate of 57μg. per 20grams of soil. The bottles containing the treated soil samples were kept in the incubator (30 ± 0.5 ^{0} C) for the duration of the experiment.

At appropriate intervals, mudflat soils were sampled and was Soxhlet-extracted in triplicate (20grams each) with analytical grade hexane (250ml). for 8 hours followed by dichloromethane for another 8 hours. The dichloromethane was evaporated to dryness, the residue redissolved with the hexane phase, and then concentrated to about 5ml. Sulfur was removed by treatment with mercury, and

finally the extract concentrated for column chromatography. Florisil column chromatography (5% deactivated with distilled water) was used for the clean-up by elution with solvents of increasing polarity (70ml of AR grade hexane, 50ml of AR grade hexane/ dichloromethane(70:30) and finally 40ml of AR grade dichloromethane). The combined elutes were collected and then concentrated using rotary evaporator to a final volume of 5ml. This extract was directly analyzed by GCMS.

A shimadzu GC 17A gas chromatography interfaced to a QP-5000 mass spectrometer was used for the quantitative analysis of the lindane. A HiCap OV 1701 fused silica capillary column (25m x 0.2mm i.d) was programmed from 60°C to 100 °C at 5 °C /min, held at 100 °C for 10min, and from 100 °C to 230 °C at 8 °C /min. The ion source and analyzer were maintained at 250 °C. Helium was used as the carrier gas at a flow rate of 40cm^3/min. Ijection volume was 2µL and the splitless mode was used. The ionization potential was 70eV. Lindane was quantified using selected ion monitoring (m/z: 111, 181, 183 and 219). Calibration was carried out using external standards. The mean recovery rates of lindane from mudflat soil were 93%. Using samples spiked with 20 µg lindane, standard deviations of the methods employed was found to be less than 11% for mudflat soil samples.

The microbial biomass was determined in accordance to the method of Jenkinson and Powlson (1975). The soil were put through a 2.5mm sieve. Eight portions of soil each containing 25g moist soil were placed in glass beakers (100ml). Four portions were fumigated with $CHCl_3$ (purified by shaking AR grade $CHCl_3$ three times with concentrated H_2SO_4 , then washed three times with distilled water and dried over anhydrous K_2CO_3) and four left unfumigated. The fumigation was conducted in a desiccator maintained in a alcohol-free $CHCl_3$ atmosphere for 24 hours, at the end of which, the soils were transferred to another clean and $CHCl_3$-free desiccator. $CHCl_3$ vapour from the soils were then removed by repeated evacuation in the desiccator by an aspirator followed by a high vacuum oil pump.

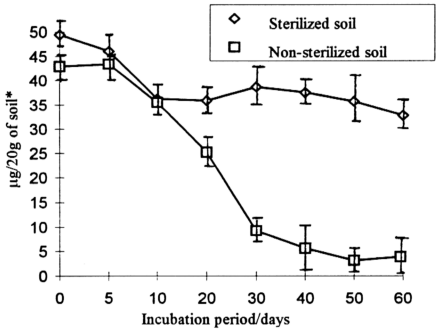
Each portion of fumigated soil was inoculated with 2.5g of moist unfumigated soil mixed evenly by spatula. After inoculation 10ml of distilled water was added to each soil. The unfumigated portions of soil were not inoculated. The portions of soil were incubated for 21days at 30^{0}C and CO_2 evolution measured. Soil from each beaker were transferred into wide neck glass bottle (500ml). A small glass sample bottle (18ml) contained 10ml of 0.25M of NaOH were placed in centre of the bottle. The wide neck bottle were closed tightly. Blank incubation in which the bottle contained 10ml of NaOH, soil were included in this experiment.

At the end of the incubation period, 10ml NaOH was made up to 25ml with distilled water and placed in a conical flask (100ml) and a few drop of fenolthelin (prepared by dissolving 0.5g of fenolthelin in 50ml methanol) and 2ml

of 1M $BaCl_2$ was added. The solution was then titrated with 0.2M HCI and the amount of CO_2 evolved during incubation calculated from the volume of acid required to neutralize the alkali.

Results and Discussion

The lindane disappeared more rapidly from non-sterilized soil compared to the sterilized soil (Figure 1). Less than 3.9µg per 20grams of soil were detected in non-sterilized samples 60 days after application compared to 32.8µg per 20grams from sterilized soil at the end of the study. The greater loss of the insecticide from non-sterilized soil indicated that there was active participation of the microflora in the degradation of lindane.



* Mean of triplicate estimations

Fig 1. Persistence of lindane in sterilized and non-sterilized mudflat soil from Kuala Selangor.

The half live of lindane in non-sterilized soil was estimated to be 20.3 days. These results suggest the establishment, of a microbial population which is able to degrade lindane and indicate that biodegradation is one of the most important factors governing the persistence of lindane in soil.

Previous observation by Macrae *et al.* (1967) based on the biodegradation of lindane in submerged soils also showed that lindane disappeared more rapidly

from non-sterilized soil compared to sterilized soil. Only trace amounts were detected in non-sterilized soil samples 70 days following application, while more than 65% of the applied insecticide were still present in sterilized soils at the end of the same period. In another study by Macrae *et al.* (1969), an anaerobic bacterium isolated from flooded soils, a species of *Clostridium* was observed to be responsible for the biodegradation of lindane in a flooded soil. Only 0.5% of the insecticide present at 1 hour of incubation was detected after 27 hours incubation with the microorganism. Hetrick *et al* (1957)and Lichtenstein *et al.*,(1960) reported that for flooded soil, the anaerobic species of the soil microflora were more active than aerobic species in the degradation of lindane. With the exception of a few millimeters of surface soil, the bulk of the submerged soil becomes anaerobic soon after flooding and anaerobic species of the soil microflora became dominant under this condition.

The biomass content of mudflat soil calculated from the increase in CO_2 evolution caused by fumigation with $CHCl_3$ is shown in Table 1.

Table 1. Biomass content of mudflat soil.

	CO_2-C evolved mg/50g soil*			
	Untreated soil 0-10 days	Untreated soil 10-20days*	Fumigated soil* 0-10days	Biomass* mgC/50g soil
Mudflat soil	13.62	5.75	16.68	191.85
S.D.	1.6	0.4	0.9	31.2

* Mean of four determinations.
S.D. : Standard deviation

The initial respiration rate and biomass (13.62 CO_2-C evolved mg/50g soil and 191.85mgC/ 50g of mudflat soil respectively), indicated a significant microbial population in the mudflat soils. The observed microbial biomass was also found to be to be significantly higher than those observed in microbially-active agricultural soils (Jenkinson and Powlson, 1975). This observation is consistent with the earlier observation of the rapid loss of lindane from nonsterilized soil compared to sterilized mudflat soil.

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Distribution of [^{14}C]- DDT and its Metabolites in a Model Mudflat Ecosystem.

Introduction

DDT is one of the most popular organochlorine insecticide ever existed. In Malaysia DDT was widely used in late 1960's and 1970's, particularly in the Malaria Eradication Program implemented in 1967. Although the use of DDT in Malaysia has been restricted, DDT residues are still detected in almost every survey in Malaysia (Tan *et al.* 1991). Although the persistent nature of DDT is well established, it has also been recognized that its behavior in the tropical environment may differ significantly compared to temperate countries. For example the more rapid volatilization of DDT in the tropics was illustrated by the recorded half life of this insecticide as 10 years in the temperate zone compared to between 6 months to 1 year in the tropical zone (Varca and Magallong, 1994).

In this investigation the distribution of [^{14}C]DDT, in a model mudflat ecosystem comprising of estuarine water, sediment and cockles (*Anadara granosa*) exposed to a sublethal concentration of the insecticide was examined for a period of 21 days.

Materials and Methods

Estuarine water, sediment and cockles (*anadara granosa*) used in this experiment were collected from the mudflats of Kuala Selangor, Peninsular Malaysia, and maintained in fibre-glass for a period of 40 days prior to the commencement of the experiments.

Mudflat soil was analyzed to be a mixture of gravel, sand, silt and clay with the following particle size distribution, expressed as percentage(%) of total dry weight : 2.0mm(0.1%), 1.0-2.0mm(0.4%), 0.5-1.0mm(1.1%), 0.25-0.5mm(16.7%), 0.125-0.25mm(15.3%), 0.063-0.125mm(30.9%), 0.063mm(35.5%). Organic content was determined as loss on ignition of 20g of freeze-dried sediment for three hours at 550^{0}C. A mean value 8.9% of dry weight was obtained.

The model ecosystem were established in 50L glass tanks with aeration (dissolved oxygen concentration averaged 6.8 mg/L). Some physico-chemical properties of the estuarine water were as follows (mean value) : temperature 25.1^{0}C ; alkalinity, 115mg $CaCO_3$/L ; pH 8.6 and Salinity 31.0 ppt. Prior to the experiment estuarine water , sediment and cockles were also analyzed for the possible presence of DDT, none was detected.

^{14}C-DDT , labeled in the phenyl rings, with a specific activity of 24.95 mCi/mmol and a radiochemical purity of 98% (as determined by TLC) was provided by the International Atomic Energy Agency, Vienna. The ^{14}C-DDT was dissolved in pesticide grade acetone (100ml) to give a solution with 4.25μCi/mL and containing 60.35μg/mL of the insecticide.

The model ecosystem was prepared by adding an appropriate quantity of this stock solution into glass tanks (50L) containing estuarine water (20L), sediment (8cm depth) and cockles (50 in number). The addition of the pesticide was accompanied with stirring to ensure uniform mixing and resulting in a ^{14} C-DDT concentration of 3 μg/L. To facilitate the sampling of sediments, glass vials filled with sediments were preintroduced during the period of acclimatisation. Water, sediments and cockles were randomly sampled in triplicate prior to the addition of [^{14} C]DDT, just after addition of the insecticide and at intervals of 3hr, 24hr, 48hr and 72hr, as well as 7, 14 and 21 days.

Extraction, subsequent analysis and counting were conducted in triplicate for water, sediment and tissue samples. Water samples were filtered, extracted with hexane and then directly counted in the liquid scintillation counter (LSC). Freeze-dried sediment samples were Soxhlet-extracted with hexane for 8hr. followed by dichloromethane for another 8hr. Cockles, removed from the tank were rinsed with tap water, deshelled and then freeze dried. After maceration and filtration, the tissues were soxhlet-extracted with hexane for 8hr.

The water, sediment and tissue extracts were evaporated to dryness at 50°C with N_2 gas and reconstituted with hexane (1mL). A portion of the concentrated extracts were counted in the LSC. Identification of metabolites was achieved by TLC with the aid of standard solutions of DDT, DDD, DDE and DDMU. The precoated TLC (silica gel 60GF-254) were developed in a mixture of hexane : dichloromethane (4:1) and visualised under UV radiation. Quantification was achieved by counting the recovered compounds obtained following preparative TLC.

Radioactivity measurements were carried out in a Beckman LS 6000SE. Radioactivity associated with bound residues was determined by combustion analysis of extracted sediment and tissue samples in a Biological Oxidizer 0X400. Recoveries of ^{14}C as ^{14}C-carbon dioxide from sediment and tissue samples fortified with known amounts of ^{14}C-DDT (2μL of the stock solution) immediately prior to combustion were not less than 91%. The counting efficiency was corrected, and the accuracy was controlled to below 5% variation.

Results and Discussion

The percentage of applied ^{14}C activity in water is shown in Table 1.

Exposure Period	O hr.	3 hr.	24 hr.	48 hr.
% of Applied ^{14}C Activity	96.9	47.6	10.6	0.4

Table 1. Detectable [^{14}C]DDT in water.

It is evident from Table 1 that the maximum activity in water was observed immediatly following addition. At the end of 48 hours only 0.4% of the applied radioactivity remained in water. At the end of 3 days, ^{14}C activity in water approached background levels. ^{14}C activity in water was attributed to the parent compound by TLC. No metabolites were detected (Table 2).

		0hr.	3hr.	24hr.	48hr.	72hr.	7days	14days	21days
Water	DDT	+	+	+	+	-	-	-	-
	DDE	-	-	-	-	-	-	-	-
	DDD	-	-	-	-	-	-	-	-
	DDMU	-	-	-	-	-	-	-	-
Sediment	DDT	+	+	+	+	+	+	+	+
	DDE	-	-	+	+	+	+	+	+
	DDD	-	-	-	-	-	-	-	+
	DDMU	-	-	-	-	-	-	-	-
Cockles	DDT	+	+	+	+	+	+	+	+
	DDE	-	-	-	-	-	+	+	+
	DDD	-	-	-	-	-	-	-	+
	DDMU	-	-	-	-	-	-	-	-

+ detected : - undetected

Table 2. Screening of [^{14}C]DDT and its metabolites in water, sediment and cockles

At the end of the study period, 85-87% of the applied radioactivity was detected in the sediment (Figure 1). The low water solubility of DDT and the ability to accumulate in organic matter-rich sediment are possible contributing factors to this obsevation (Young *et al.*, 1977).

The level of [^{14}C]DDT in the sediment increased after 7 days following application and the highest level (0.10μg/g) was observed at the end of 21 days after application. These appears to be an increase in the bound residues at the

Figure 1: Distribution of 14C-DDT residues in the model mudflat ecosyatem.

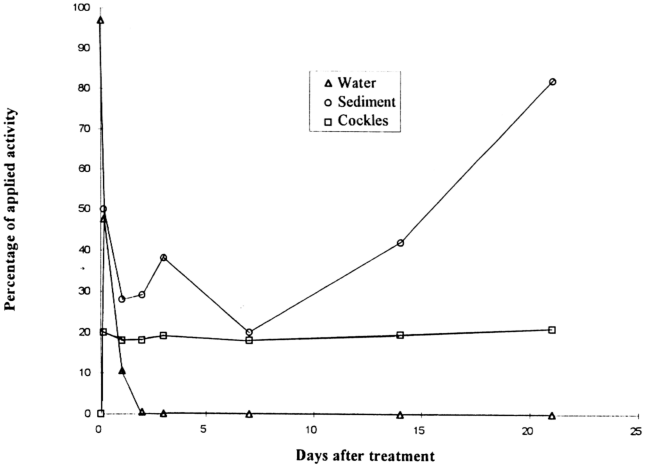


Figure 2: 14C-DDT residues in sediments

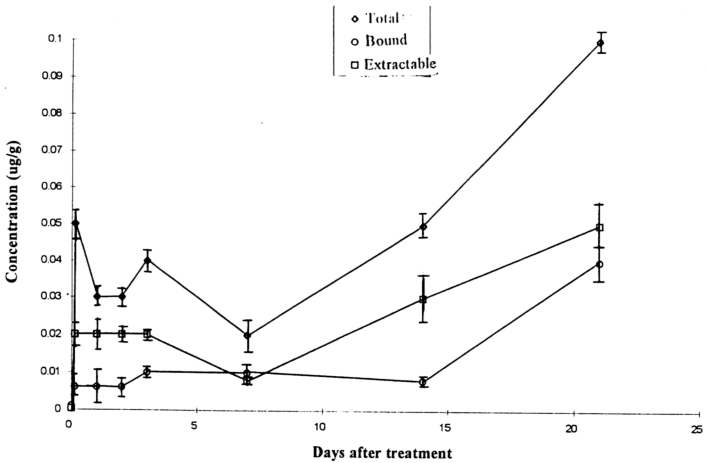
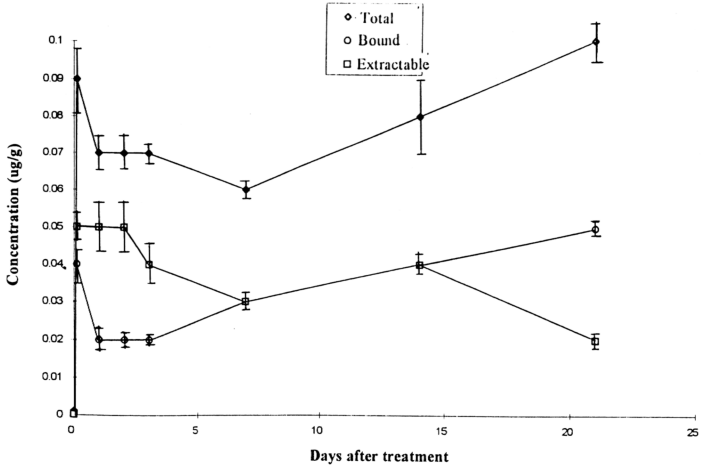


Figure 3: 14C-DDT residues in cockles



expanse of extractable residues in the sediment. Hence, bound residues which amounted to 20% of the total residues in the sediment after 24 hours increased to 40% at the end of 21 days. By contrast, extractable residue after 24 hours was 67% of the total residues in the sediment, decreasing to 50% at the end of 21 days.

In the sediment samples, DDE was the major metabolite detected (0.003-0.008 μg/g). It was first observed at the end of 24hours. DDD, on the other hand was first observed 3 weeks after commencement of the study (0.002 μg/g). DDT was present in all the sediment samples (0.004-0.020 μg/g).

About 20-22% of the total applied activity was detected in the cockles at the end of 21days. The levels in the cockles appear to remain fairly constant over the whole study period. It is evident particurlarly at 21 days much of the residues in the cockles were of the bound variety (50% bound and 20% extractable relative to total residues in cockles). In cockles DDE was first observed at the end of 7 days (0.001-0.002 μg/g). while DDD was detected at the end of three weeks (0.001μg/g). As with the sediment samples, DDT was present in all the tissue samples (0.001-0.009 μg/g). DDMU was not detected at all during the course of the study.

DDT is known to be absorbed, bioaccumulated and translocated in aquatic invertebrates (Fleming *et al.*, 1962; Kerr and Voss, 1973 and Tatsukawa, 1992). The principal feeding process of cockles is filter-feeding, whereby suspended microscopic particles are removed from water passing over the gills. The particles can be zooplankton, phytoplankton and organic detritus (Elder and Collins, 1991). DDT can be absorbed in organic detritus and can be taken by cockles in filter-feeding process and bioaccumulated in the fat.

Hence results from this study illustrated the movement of DDT to the various components of the mudflat ecosystem. These was rapid partitioning of the chemical from the water phase into both sediments and cockles. While the levels in sediment increase with time those observed in cockles appear to remain constant over the study period.

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