CHAPTER 3

DISTRIBUTION OF ^{14}C- DDT AND ITS METABOLITES IN A MODEL MUDFLAT ECOSYSTEM.

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

3.1 General

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 to public health use only, DDT residues are still detected in almost every survey in Malaysia [1]. While 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 [2]

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

3.2 Reagents and apparatus

The following reagents and apparatus were used:

a) [^{14}C]DDT	IAEA (International Atomic Energy Agency)(99.5% purity)
b) DDT	IAEA (International Atomic Energy Agency)(99.5% purity)
c) DDE	IAEA (International Atomic Energy Agency)(99.5% purity)
d) DDD	IAEA (International Atomic Energy Agency)(99.5% purity)
e) DDMU	IAEA (International Atomic Energy Agency)(99.5% purity)
f) PPO	BDH, analytical grade (98.5% purity)
g) POPOP	BDH, analytical grade (98.5% purity)
h) Toluene	BDH, analytical grade (99.5% purity)
i) Triton X-100	BDH, analytical grade (98.5% purity)
j) Silica gel	MERCK, Kielselgel 60 GF_{254} 7730
k) Biological oxidizer	HARVEY, OX 400
l) Liquid scintillation counter	BECKMAN, LS 6000SE

3.3 Preparation of a model mudflat ecosystem.

Estuarine water, sediment and cockles (*Anadara granosa*) collected from Kuala Selangor were maintained in a fibre-glass

tanks with aeration for a period of one and half months prior to the commencement of the experiments.

The model mudflat ecosystem was established in a 50 litre glass (60cmx30cmx30cm) with aeration (dissolved oxygen concentration averaged 6.8mg/L) with approximately 6 kg of sediment (8cm depth), cockles (100 in number, biomass 60g) and 9 litre of estuarine water. For collection of sediment samples, small vials were perintroduced into the tanks. Hence, sampling of sediment was conducted by removing the glass vials filled with sediment at the appropriate time intervals with minimal disturbance to the model ecosystem. Mudflat soil was analyzed to be 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 and 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 of 4.25μCi/mL and containing 60.35μg/mL of the insecticide. The model ecosystem was prepared by adding the appropriate quantity of the stock solution into the water column, accompanied with stirring to ensure uniform mixing and resulting in a [^{14}C]DDT concentration of 3μg/L.

3.4 Preparation of thin layer chromatography

Thin Layer Chromatographic plates used in this study were prepared in the laboratory by the following procedure. Glass plates (20x20cm) were initially cleaned with acetone to remove dust and grease. A slurry of 60g silica gel (Kieselgel 60 GF_{254}) in water (120ml) was shaken vigorously for one minute and then spread onto the plates by using a TLC spreader. The thickness of the silica gel used was 1.0mm.

The prepared plates were dried at room temperature for 30 minutes before being activated by heating in the oven at 110^{0}C for at least one hour. The TLC plates were developed in a mixture of

hexane : dichloromethane (4:1) and visualised under UV radiation. UV-active bands corresponding to the parent compound and metabolites were removed and the residues dissolved in hexane.

3.5 Sampling and sample analysis.

3.5.1 Estuarine water

Estuarine water (100ml) were sampled in triplicate, just before pouring into the tanks as well as during the following times: 3 hours, 24 hours, 48 hours, 72 hours, 7 days, 14 days and 21days. The extraction was carried out as described in Section 2.5.1. The aliquots of hexane extracts were evaporated to dryness at 50^0C with N_2 gas and reconstituted with hexane (1ml). The ^{14}C-activity of half of the extract was determined in the Beckman liquid scintillation counter. The other half of the extract was analyzed by preparative TLC. The scintillation cocktail used was a mixture of PPO, 5.5g ; POPOP, 0.1g ; toluene, 667ml ; triton X-100, 330ml. Identification of the metabolites was achieved by analytical TLC (silica 60GF-254) with the aid of standard solutions of DDT, DDD, DDE and DDMU. Quantification was achieved by determining the [^{14}C]activity of recovered compounds obtained following preparative TLC.

3.5.2 Sediment

Sediment samples were collected by pooling the sediment in the preintroduced vials removed with a metal forceps with minimal disturbance to the ecosystem. Sampling was undertaken upon commencement of the experiment as well as at the following time interval: 3 hours, 24 hours, 48 hours, 72 hours, 7 days, 14days and 21days. The pooled sediment samples were wrapped with precleaned aluminum foil and maintained at -20^{0}C. For the analysis, sediment samples were removed from the freezer and allowed to warm to room temperature. Excess water were removed by suction filtration followed by freeze-drying.

Triplicate freeze-dried sediment samples (20g) were extracted and analyzed as described in Section 2.5.2. The aliquots of hexane extracts were concentrated to 1ml and half of this was analyzed for ^{14}C- radioactivity in the LSC. The other half of the extract was analyzed by preparative TLC. Radioactivity associated with bound residues was determined by combustion analysis of the extracted sediment in a Harvey biological oxidizer. Recoveries of ^{14}C as [^{14}C]carbon dioxide from sediment fortified with known amounts of [^{14}C]DDT corresponding to 2μL of stock solution immediately prior to combustion were not less than 91%. The counting efficiency was corrected, and the accuracy was

maintained to below 5% variation. Identification and quantification of metabolites was achieved by TLC as described in Section 3.4.

3.5.3 Tissue

Cockles were removed by metal forceps with minimal disturbance to the to ecosystem at 0 hour, 3 hours, 24 hours, 48 hours, 72 hours, 7 days, 14days and 21days time interval. Pooled samples (8-10 animals per replicate) were wrapped with precleaned aluminium foil and were maintained at -20^{0}C prior to analysis. For the analysis, cockles were removed from freezer and deshelled using a stainless steel tweezer and spatula. The deshelled cockles was rinsed thoroughly with distilled water and excess water were removed by suction filtration. The cockles tissue were cut into small pieces with the aid of a stainless steel knife and scissors and then freeze-dried.

Freeze-dried tissue (5-10g) was extracted in accordance to the procedures described in Section 2.5.3. The extracts were evaporated to dryness and reconstituted with hexane (1ml). Half of this extracts were counted in the LSC. The other half of the extract was analyzed by preparative TLC. Radioactivity associated with bound residues was determined by combustion analysis of the

extracted tissue in a BO. Identification and quantification of metabolites was achieved by TLC as described in Section 3.4.

3.6 Results and Discussion

The [^{14}C]activity in water expressed as percentage of applied ^{14}C activity in water is shown in Table 3.1

Exposure Period	O hr.	3 hr.	24 hr.	48 hr.
% of Applied ^{14}C Activity*	96.9±4.8	47.6±2.4	10.6±0.6	0.4±0.02

* Mean of triplicate determination ± standard deviation.

Table 3.1 [^{14}C]DDT in water.

It is evident from Table 3.1 and Figure 3.1 that the maximum activity in water was observed immediately following addition of the chemical. 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 as evidenced by TLC. No metabolites were detected (Table 3.2).

A previous study by Kale and co-workers with a model ecosystem consisting of water, sediment, and clams exposed to ^{14}C-DDT, 79.77% of the applied activity was detected in the water column at the beginning of the experiment. ^{14}C activity reduced to

52.60% at the end of 2 hours and was only 1.13% of the applied activity at the end of 3 days [3]. Similarly, Matin and co-workers observed a reduction of [^{14}C]activity in the water column from 94% at the beginning of the experiment to 1.5% at the end of the study period (30 days) [4].

		0hr.	3hr.	24hr.	48hr.	72hr.	7days	14days	21days
Water (µg/L)	DDT	2.92	1.44	0.32	0.01	-	-	-	-
	DDE	-	-	-	-	-	-	-	-
	DDD	-	-	-	-	-	-	-	-
	DDMU	-	-	-	-	-	-	-	-
Sediment (µg/g)	DDT	-	0.020	0.009	0.009	0.007	0.008	0.006	0.004
	DDE	-	-	0.003	0.007	0.006	0.004	0.008	0.005
	DDD	-	-	-	-	-	-	-	0.002
	DDMU	-	-	-	-	-	-	-	-
Cockles (µg/g)	DDT	-	0.009	0.008	0.008	0.006	0.002	0.001	0.001
	DDE	-	-	-	-	-	0.002	0.001	0.002
	DDD	-	-	-	-	-	-	-	0.001
	DDMU	-	-	-	-	-	-	-	-

-: undetected

Table 3.2. [^{14}C]DDT and its metabolites in water, sediment and cockles.

The distribution of [^{14}C]activity in water, sediment and cockles expressed as percent of applied activity as a function of period of exposure is given in Figure 3.1. [^{14}C]activity in the sediment ranged between 22 - 50% of the applied activity in the first 7 days of the study. Thereafter an increase in [^{14}C]activity was observed with a maximum of 85-87% of the applied radioactivity at the end of 21 days.

The distribution of [^{14}C]activity in sediments in the forms of total, bound and extractable residues as a function of period of exposure is given in Figure 3.2. The level of total [^{14}C]activity in the sediment expressed in concentration terms, ranged between 0.02 - 0.05μg/g in the first 7 days of the experiment, thereafter increasing to a maximum of 0.10μg/g at the end of 21 days following application. Furthermore, as the experiment progressed there appears to be an increase in the bound residues at the 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 ranging from 0.003-0.008 μg/g (Table 3.2). DDE was first observed at the end of 24hours. DDD, on the other hand was first detected 3 weeks after commencement of the study amounting to 0.002 μg/g. DDT was present in all the sediment samples ranging from 0.004-0.020 μg/g. DDMU was not detected at all.

The presence of DDT in the sediment was more significant than in water, consistent with its liphophilic properties, characteristic of OC insecticides. In numerous studies sediment were found to contain significantly high residues of DDT and its metabolites compared to the levels in water [5,6]. A summary of pesticide residue data involving 40 water samples and 84 sediment samples between 1968-1969 in Ontario showed that DDT levels in sediments averaged 80μg/kg compared to levels in the water column which averaged 19ng/L [7]. The sediments used in this study are very rich in organic matter (8.9% on a dry basis), and this can act as a very good reservoir for the OC pesticide. Choi and Chan also demonstrated that humic substances, which are important components of organic matter in sediment, play a major role in the accumulation of chlorinated hydrocarbons [8]. Bailey and White have also shown the importance of OM content of the soil in influencing pesticide sorption equilibria [9]. The highly liphophilic insecticide DDT tend to partition into sediment from the water phase and are not readily leached through the soil column [10]. Recent studies have also shown an increase in [^{14}C]activity in the sediment as a function of time when labelled DDT was introduced in the water column [3,4,11].

Many studies have shown that sediment in both the freshwater and marine environment can act as reservoir for persistent pesticides such has DDT [8,9,12,13]. In a study conducted by Hindin and co-workers, DDT residues in the bottom sediment ranged between 56 - 144 mg/kg while the metabolite DDD averaged 95mg/kg in the Columbia river basin [12].

The gradual degradation of DDT in the environment is exemplified by a study by Frank and co-workers who reported that the DDT level in sediment collected from Lake St. Clair in 1970 decreased from 6.6ppb to 2.4ppb in 1974, 85% of which was in the form of TDE or DDE [13].

[^{14}C]activity in the cockles appear to remain fairly constant over the whole study period ranging from 20%-22% of the total applied activity (Figure 3.1). Total, bound and extractable residue in cockles as a function of time is given in Figure 3.3. It is therefore evident that at end of 21 days most of the residues in the cockles were of the bound variety. Extractable residues ranged between 0.001 and 0.05μg/g from the time of application to 48 hours, thereafter decreasing to 003μg/g at the end of 7 days. Extractable residues comprised 0.02μg/g of the total residues at

the end of the study period. Bound residues ranged between 0.0002 and 0.02μg/g at the beginning of the experiment and 72 hours, thereafter an increase was noted to a maximum of 0.05μg/g relative to the total residues at the end of the study. In the cockles, DDE was first observed at the end of 7 days ranging from 0.001-0.002 μg/g, while DDD was detected at the end of three weeks (0.001μg/g) (Table 3.2). 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.

The ability of aquatic organism to concentrate OC chemical many times above levels found in water has been widely demonstrated and documented. Hence, DDT and its metabolites have been detected in a various of aquatic organism including Crustaceans, Mollusc, Mussel and fish [14-18]. In a study conducted by Zhong and Chen involving bioaccumulation of DDT in marine shrimp, clam and tilapia, DDT was found to be distributed among various organs in different proportions. Bioconcentration factors in shrimp organs were 8512.3(digestive gland), 901.00(pereopod), 447.76(eye), 326.51(stomach), 287.36(carapace), 286.71(gill), 254.46(intestine) and 99.83(muscle). DDE was found to be the major metabolite amounting to 61.02% of the total radioactivity [19].

Bioconcentration factors in clam organs ranged between 10213 to 89929, the highest being in the gill [20]. In tilapia the highest bioconcentration factor were found to be 48477 in the liver while the lowest was in muscle tissue (686). DDD(6.25% of applied activity) was found to be a major metabolite, followed by DDE(1.95%), DDA(1.76%) and DDMU(0.98%) [21].

Bevenue observed that the uptake of DDT by aquatic invertebrates was directly proportional to the concentration of insecticide in the medium and to the duration of exposure [22]. Similar observations were also made by Derr and Zabik with DDE and the aquatic organism Chironomid (*Chironomus tentans*) [23]. Working with 10 species of invertebrates, Johnson and co-workers also found that the mean DDT residues were 0.62, 1.25 and 2.00 ppb, respectively, at 1,2 and 3 days exposure to 0.1 ppb pesticide in the water using a continuous flow system [24]. Hamelink and co-workers reported that following an initial application of DDT in an experimental area (farm pool, artificial pools, aquarium), levels of DDT in water declined with a corresponding increase in the levels detected in several invertebrates species [25]. O'Brien have also noted the ability of many invertebrates to metabolize DDT [26].

Significant levels of persistent pesticides, particularly DDT and its metabolites, in aquatic invertebrates was only observed sometime after pesticide treatment [27]. DDT is known to be absorbed, bioaccumulated and translocated in aquatic invertebrates [28-30]. 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 [31]. DDT can be absorbed in organic detritus and can be taken by cockles in filter-feeding process and bioaccumulated in the fat.

Hargrave and Phillips observed the presence of DDT and its metabolites in several aquatic invertebrates collected from St. Margarets Bay [14]. Most of the residues were p,p'-DDE, except in the Echinodermata, Pelecypoda and Polychaeta, where p,p'-DDT was also present; p,p'-DDD was found only in the pelecyponds. The mussel *Mytilis edulis* sampled in this study contained residues of DDE, DDT and DDD.

Hence results from this study illustrated the movement of DDT to the various components of the mudflat ecosystem. There was rapid partitioning of the chemical from the water phase into both sediments and cockles, consistent with the liphophilic

characteristic of DDT [10,28]. The levels of the chemical in sediment increased with time, while those observed in cockles appear to remain fairly constant over the study period.

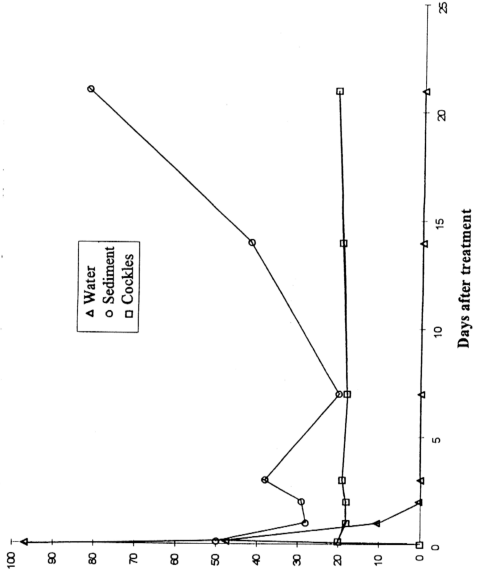

Figure 3.1: Distribution of [^{14}C]activity residues in the model mudflat ecosystem

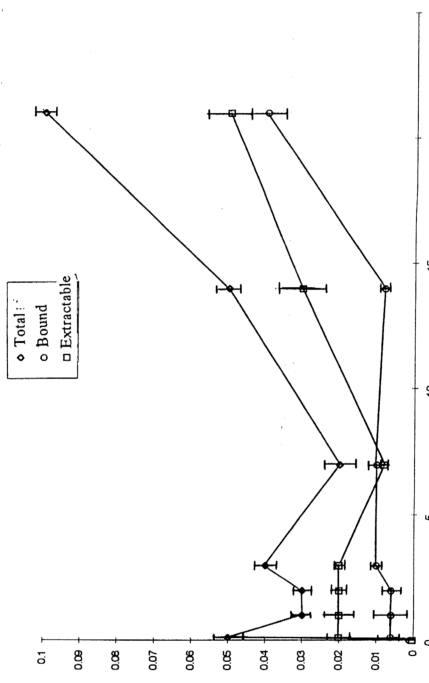

Figure 3.2 : [^{14}C]activity residues in sediments

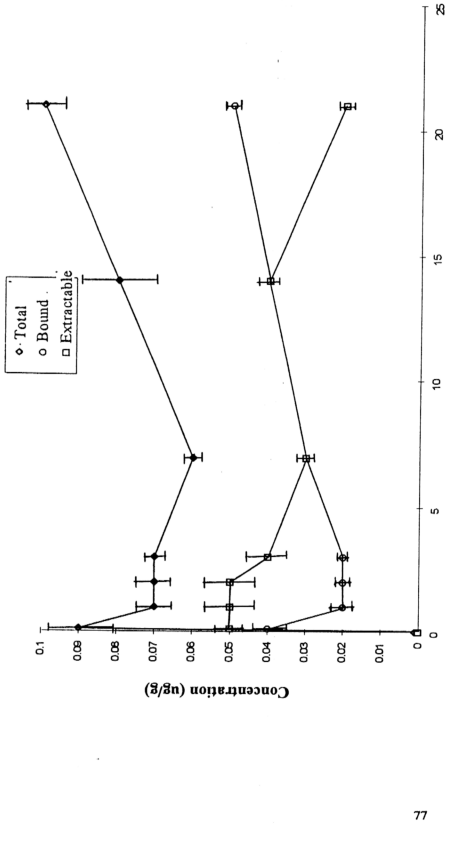

Figure 3.3 : [^{14}C]activity residues in cockles

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