

Distribution of ^{14}C -DDT and its metabolites in a model mudflat ecosystem

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Abstract. The distribution of ^{14}C -DDT and its metabolites in a model mudflat ecosystem comprising water, sediment and cockles (*Anadara granosa*) was investigated for a period of 21 days under laboratory conditions. The rapid decrease in ^{14}C -DDT concentration in water coincided with a corresponding increase in concentration of the chemical in sediment which reached a maximum of 84.4 % of the applied ^{14}C activity at the end of the study period. The proportion of extractable residues in the sediment was also noted to increase gradually with time at the expense of bound residues. The ^{14}C activity in cockle tissue was relatively constant, averaging 19.8 % of the total applied activity. At the end of the 21-day study, 71 % of the residue in the tissue was bound. DDT was the major chemical identified in water, sediment and cockle tissues. Traces amounts of DDE and DDD were also detected in the later part of the study.

Abstrak. Taburan ^{14}C -DDT dan metabolit-metabolitnya dalam suatu ekosistem lumpur model yang mengandungi air, enapan dan kerang (*Anadara granosa*) telah dikaji selama 21 hari dalam makmal. Penurunan deras kepekatan ^{14}C -DDT dalam air diiringi dengan kenaikan kepekatan bahan kimia tersebut dalam enapan, dimana nilai maksimum sebanyak 84.4 % daripada jumlah keaktifan ^{14}C yang dibekal diperhatikan pada penghujung tempoh kajian. Disamping itu, nisbah sisa yang dapat diekstrakkan dalam enapan berbanding sisa terikat didapati meningkat dengan masa. Aktiviti ^{14}C dalam tisu kerang didapati tidak berubah secara signifikan, dengan nilai purata sebanyak 19.8 % daripada jumlah keradioaktifan yang dibekal. Pada penghujung 21 hari, sisa terikat merupakan 71 % daripada jumlah sisa dalam tisu. DDT merupakan kimia utama yang dikenalpasti samada dalam air, enapan atau tisu kerang. Amaun surih DDE dan DDD juga dapat dikesan pada akhir akhir kajian.

Introduction

DDT has been identified as one of the most persistent global contaminant and is known to spread throughout the globe including to remote areas such as the North Pole through long-range atmospheric transport [1]. The chemical has been banned in all developed countries since the 1970s. The use of DDT has also been banned or restricted in increasing numbers of developing countries, most of which are located in the tropical zone [2].

As is characteristic of the organochlorine (OC) class of pesticides, the rate of degradation of DDT in the environment is slow. A $t_{1/2}$ of 10

years has been estimated for DDT in the temperate zone whereas in the tropical zone the $t_{1/2}$ of the chemical has been recorded as 6 months-1 year [3] attributed predominantly to the more rapid volatilization of the chemical upon application in the tropics [4].

Although DDT has been banned for use in Malaysia for more than a decade, monitoring surveys conducted over the years have consistently shown the presence of DDT and its metabolites, particularly DDE and DDD in both abiotic and biotic components of the environment [5,6]. While the continued presence of the chemical in the environment is attributed to its persistent characteristic, results of several

monitoring surveys also indicate the possibility of continued, sporadic illegal applications.

Residues of DDT and its metabolites have also been observed in the marine environment [7, 8]. The mudflats in Malaysia, particularly along the western coastline of Peninsular Malaysia are commonly used for the culture of marine bivalves, including *Anadara granosa*. The principal feeding process of bivalves is by filter feeding, whereby suspended microscopic particles are removed from water and passed over the gills. Hence, lipophilic pollutants such as DDT can be absorbed in organic detritus, taken up by the organism and bioaccumulated in fatty tissues.

The present investigation elucidated the behavior and fate of persistent OC chemicals, as characterized by DDT, in the tropical environment. A model mudflat ecosystem was selected comprising water, sediment and cockles (*Anadara granosa*) which was exposed to ^{14}C -labeled DDT and the partitioning of the chemical between the components of the ecosystem monitored.

Experimental

Test chemicals

^{14}C -DDT labeled at 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 (IAEA), Vienna. Non-labeled DDT, DDE and DDD were also provided by IAEA (> 98 % purity). ^{14}C -DDT was dissolved in acetone to give a solution with an activity of 4.25 $\mu\text{Ci/ml}$ which corresponded to 60.35 $\mu\text{g/ml}$. All solvents used were of pesticide-grade.

Radioanalysis

Quantitative ^{14}C measurements were made with a Beckman LS 6000 liquid scintillation counter (LSC), while insoluble ^{14}C residues were determined by combustion analysis with a Harvey OX 600 biological oxidizer. The scintillation cocktail used was a mixture of PPO (5.5 g), POPOP (0.1 g), toluene (667 mL) and triton X-100 (330 mL).

Preparation of the model mudflat ecosystem

Water, sediment and cockles were collected from the mudflats of Kuala Selangor, Malaysia. The model ecosystem was established in a all-glass aquarium tank (90 cm x 70 cm x 40 cm) filled with seawater (40 L), sediment (6 Kg, 4 cm depth) and 150 cockles of approximately equal size. Sample vials inserted into the sediment were evenly distributed to facilitate the sampling of sediment with minimum disturbance to the ecosystem. The model ecosystem was gently aerated resulting in a dissolved oxygen content which averaged 6.8 mg/L throughout the experiment.

The sediment was characterized 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 %). The organic content of the sediment was determined as loss on ignition of 20 g of dried sediment for 3 hours at 550°C. A mean value of 8.9% of dry weight was obtained. Selected physicochemical properties of the seawater were also determined (mean values): temperature, 24.4°C; alkalinity, 110 mg CaCO_3/L ; pH, 8.6 and salinity, 32.5 ppt.

Distribution and partitioning experiments

The experiments were performed in duplicate tanks. Following a two-week period of acclimatization, the appropriate quantity from the stock solution of ^{14}C -DDT was added to give a nominal concentration of 3 $\mu\text{g/L}$. The introduction of the chemical was carried out in such a manner as to ensure a homogenous solution in the water phase of the ecosystem. Triplicate samples of water, sediment and cockles were collected immediately after introduction of the chemical and at the following time intervals: 3 h, 24 h, 48 h, 72 h, 7 days, 14 days and 21 days.

Extraction procedures and analysis

Water samples (40 mL) were extracted with hexane (10 mL X 2). After drying with anhydrous sodium sulphate, the solvent was evaporated to dryness with the aid of purified N_2 .

and the residue redissolved in 1 ml hexane to be analyzed for ^{14}C by LSC.

Freeze-dried sediment samples (20 g) were soxhlet-extracted with hexane for 8 h, followed by dichloromethane for another 8 h. The solvent was then evaporated to dryness and the residue redissolved in 5 mL hexane. Sulphur was removed by treatment with mercury and finally the extract was reconstituted to 1 mL with hexane to be analyzed for ^{14}C by LSC. ^{14}C activity associated with bound residues was determined by combustion analysis of the extracted sediment samples in a biological oxidizer.

The extraction procedure and analysis for tissue samples were similar to those for sediment samples. Hence, freeze-dried tissue samples were soxhlet-extracted with hexane and dichloromethane and then concentrated. Lipids were removed by treatment with concentrated sulphuric acid and finally the extract reconstituted to 1 mL with hexane. Extractable residues were directly measured by LSC while bound residues were estimated by combustion of the extracted tissue samples.

The water, sediment and tissue extracts were also analyzed on TLC (silica gel 60F254) for the parent compound and metabolites. The plates (20 cm X 20 cm) were developed using hexane:dichloromethane (4:1, v/v). The radioactive zones corresponding to DDT, DDE and DDD were identified by co-chromatography with unlabeled reference compounds. The Rf values were as follows: 0.59 (DDT), 0.63 (DDD) and 0.66 (DDE). The corresponding bands were removed, dissolved in hexane, filtered and analyzed for ^{14}C by LSC.

Results and Discussion

As a means for the evaluation of the distribution, fate and effects of pesticides in the environment, the model ecosystem approach has provided useful information in the assessment of pesticide impact on the ecosystem [9]. In the present study, the use of radiolabeled DDT allowed for the application of trace amounts of the chemical as is usually found in ambient waters. Recoveries of ^{14}C from water, sediment and tissue samples fortified with 2 ppb of the

chemical were not less than 89 % with a variation range of 9-13 %. Recoveries of ^{14}C as $^{14}\text{C}\text{-CO}_2$ from sediment and tissue samples fortified with 2 μL of the stock solution immediately prior to combustion were not less than 91 %. The counting efficiency was corrected.

Figure 1 shows the distribution of ^{14}C activity in the model ecosystem as a function of time. Analysis of the water samples immediately following introduction of the chemical accounted for 98% of total applied radioactivity. The ^{14}C activity in water was then observed to rapidly decrease, accounting for 11 % of the applied activity at the end of 24 h and approaching background levels at the end of 48 h. Only the parent compound was detected in the water extracts.

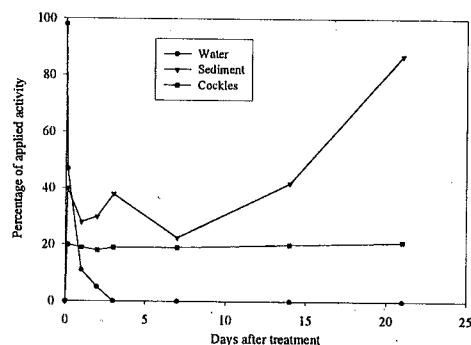


Figure 1: Distribution of ^{14}C activity residues in the model mudflat ecosystem

In the first seven days of the study, ^{14}C activity in the sediment ranged between 22.5 % - 40 % of the applied activity (Figure 1). Thereafter an increase in ^{14}C activity was observed, reaching a maximum of 87 % of the applied activity at the end of 21 days. The distribution of ^{14}C activity in the sediment expressed in the forms of extractable and bound residues is given in Figure 2. The increase in ^{14}C activity in the sediment was noted to coincide with a corresponding increase in the proportion of extractable residues at the expense of bound residues. DDT was the major compound identified in all the sediment samples. However trace amounts of DDE and DDD were also detected towards the end of the study period.

In contrast to the observed trend in water and sediment, ^{14}C activity in cockle tissue appeared to remain relatively constant

throughout the study period, averaging 19.8 % of the applied activity (Figure 1). The proportion of extractable and bound residues in the tissue expressed in $\mu\text{g/g}$ is given in Figure 3, which shows that at the end of the study period 71 % of the residue was of the bound variety. As with the sediment, DDT was the major chemical detected. DDE and DDD were also detected in trace amounts at the end of the 21-day study period.

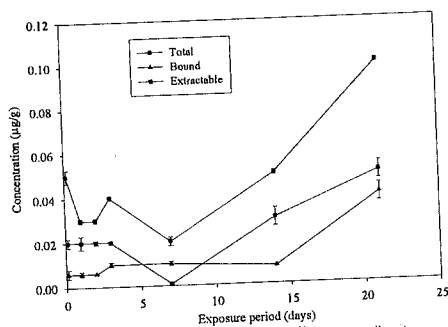


Figure 2: Total, extractable and bound ¹⁴C-DDT in sediment

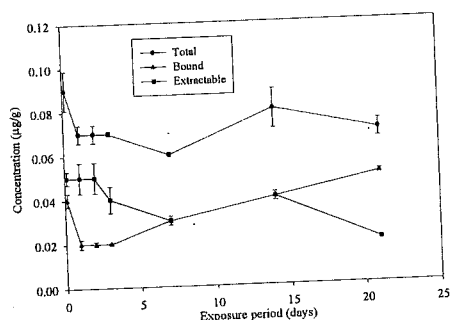


Figure 3: Total extractable and bound ¹⁴C-DDT in cockle tissues

The rapid partitioning of DDT from the water phase into sediment is characteristic of the OC class of chemicals, attributed to its lipophilic property [10,11], as demonstrated in many surveys where the levels in sediment were found to exceed those in the water column by several orders of magnitude [12,13]. The sediment used in the present study are rich in organic matter content which act as reservoir for the chemical. Previous studies have shown that the organic matter content, in the form of humic substances play a major role in the accumulation of OC chemicals [10].

The ability of aquatic organisms to concentrate OC chemicals many times above levels found in water has been widely demonstrated and documented [14,15]. Hence,

environmental surveys on OC compounds have frequently included aquatic biota particularly bivalves which are used as sentinels for monitoring pollution in the marine environment as exemplified by the mussel watch programme [14,16]. In the present study the bioconcentration factor for DDT in the cockle was estimated to be 50.

Results from the present investigation clearly illustrated the rapid partitioning of DDT into sediment and biota from the introduced aqueous solution of the chemical. The study also provided estimates of bound residues in sediment and biota which raises the possibility of such matrices acting as reservoirs for the chemical, while the extractable portions may present possible sources of DDT which may re-enter the environment. Furthermore, results from the study also emphasized the need for monitoring surveys of such chemicals to include the analysis of sediment and/or biota so as to provide a more realistic assessment of OC chemical pollution of the environment

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