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# BEHAVIOUR OF ENDOSULFAN AND LINDANE DURING SEDIMENT ELUTRIATE AND WATER SPIKE TOXICITY TESTS UNDER SALINE CONDITIONS

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## ABSTRACT

**Potential exposure to chemicals in the ecosystem can be predicted from a set of physicochemical analyses on mobility and degradation. Standard elutriate toxicity tests proposed at 1:4 (v/v) of undisturbed sediment mixed with water has been validated to be used as a conservative predictor of dredging site dissolved chemical concentrations. Sediment samples obtained from the Chantaburi estuary were spiked with endosulfan and lindane and subjected to elutriate process under 16% salinity. The average dissolution potential was as low as 0.23% for endosulfan and 1.84% for lindane indicating strong affinity for hydrophobic contaminants in the Chantaburi estuary sediment. The relationship of dose dependent endosulfan and lindane dissolution in sediment showed gradual decrease of dissolution as the spiked concentration of sediment increased, indicating mass action effect on hystresis sites. The negative non-linear power regression indicated a better relationship and the coefficients of determination ($R^2$) for endosulfan and lindane were 0.97 and 0.87, respectively. This indicated that dissolution of endosulfan and lindane into the water column from Chantaburi estuary sediment was minimal. The results of this study confirmed that after vigorous resuspension event of sediment (= elutriate), α-endosulfan was the dominant fraction followed by β-endosulfan and endosulfan sulfate. In contrast to elutriates, endosulfan compounds measured in seawater after 96 h spiking contained more β-endosulfan than α-endosulfan, thus lowering the true potential in the water column. The mean total endosulfan and lindane concentrations which remained in toxicity test matrices at 96 h were 7.52% and 15.54%, respectively. This could be due to possible loss of toxicants through volatilization and/or adsorption to the exposure vessels.**

**KEYWORDS:** Dissipation, Dissolution, Endosulfan, Lindane, Sediment elutriate.

## INTRODUCTION

Endosulfan and lindane belong to cyclodiene and cyclohexane groups of chlorinated pesticides, respectively. Technical-grade of endosulfan contains about 94% of two pure isomers, α- and β-endosulfan in the ratio of 7:3. Endosulfan is classified as an organochlorine (Guerin and Kennedy, 1992), or as non-organochlorine (Hoechst, 1993). Lindane is the γ-isomer (>99% pure) of hexachlorocyclohexane (WHO, 1991). Although the production and use of many organochlorine compounds, including endosulfan and lindane have been banned in many countries for more than 10 years (UNEP, 2002), they continue to be detected in estuarine sediments, water and aquatic biota across the world (Ramesh *et al.*, 1990; Iwata *et al.*, 1994). Residues of these

compounds can occur at levels lethal to certain species of aquatic and terrestrial animals (Coat *et al.*, 2006).

The fate of endosulfan in the environment is different for the two isomers depending on the medium where they are deposited. β-endosulfan is more persistent than its α-isomer (Antonious and Byers, 1997). Endosulfan sulphate, the main degradation product of both isomers is more persistent in the environment than its parent compounds. Lindane is more soluble in water than most of the other organochlorine compounds. Lindane is strongly adsorbed on soils that contain large amounts of organic matter. Volatilization seems to be an important route of dissipation under higher temperature conditions (WHO, 1991). Resuspension of organochlorine pesticides back to water column can happen by many processes such as sediment dredging, bioturbation, intense shipping, *etc.* Exposure analysis, in terms of concentration and the distribution of pesticides in the sediment and other compartments is instrumental in assessing the ecological risk. Standard elutriate toxicity tests proposed at 1:4 (v/v) of undisturbed sediment mixed with water has been validated to be used as a conservative predictor of dredging site dissolved chemical concentrations (USACE, 1998). The test organisms exposed to elutriate fraction using static systems for a defined duration are used to mimic exposure effects. However, the design of these systems prevents the determination of the significance of fate processes such as dissolution, dissipation and degradation within and associated environmental media. Such fate processes are predominant in nature and are likely to determine the bioavailability and hence influence the toxicity to aquatic organisms. The effects of such processes on the residence time of endosulfan and lindane and its concentration in water column have not been studied so far.

The main objective of this study was to elucidate the influence of endosulfan and lindane on their solubility and toxicity during water and sediment elutriates toxicity tests. The test system employed was static non-renewal for a period of 96 hours.

## MATERIALS AND METHODS

### Sediment sampling

The bottom sediment samples were collected from the Chantaburi River mouth estuary in Muang district, Chantaburi province, Thailand. The samples were taken by SCUBA diving. Sediment cores were collected using pre-cleaned PVC coring device (diameter: 5 cm, length: 100 cm). The cores were manually pushed to obtain around 100 cm core of sediment and composited in rigid polyethylene ice boxes lined with aluminum foils. Sediment samples were returned to the laboratory and stored at 4°C

within 2 hours of collection. Bulk quantities of seawater were sampled into pre-cleaned HDPE bottles.

**Experimental setup**

Toxicity test was conducted in approximately 2 l capacity bowl type glass chambers containing 1 l of test solution. Natural seawater with 16% of salinity was used as common diluents of toxicity tests. All test chambers were maintained without aeration during the 96 hours experimental duration. Triplicate chambers were maintained for all the treatments and were randomly placed in the test location. The experiments were conducted at ambient temperature of 29 ± 2°C and natural light: dark photoperiod of 12:12 h. Static, non-renewal acute toxicity tests were performed.

**Elutriate preparation**

Integrated core sediment samples stored under 4°C for less than 3 days was used for elutriate preparation. The 1:4 (v/v) concentration of elutriates were drained off for toxicity tests and for chemical analysis from each sediment chamber spiked with endosulfan, lindane and binary mixture of endosulfan and lindane. The method described by USACE (1998) was used in the elutriate preparation with modifications. This modification was in agreement with Lee and Mariani (1977) to simulate sediment resuspension effect. A homogenized sediment sample and overlying water were combined in a sediment-to-water ratio of 1:4 on volume basis at room temperature (28-29°C) using the method of volumetric displacement (Daniels *et al.*, 1989). The mixture was stirred vigorously for 30 min. with a probe and intermittent hand mixing. The mixture was allowed to quiescent settling for 1 h. The supernatant was then carefully removed and immediately used for testing. The required volumes of elutriates for chemical characterization were drained into pre-cleaned glass chambers within a minimal time period and none of the situations exceeded for more than 3 h. All samples were stored at 4°C for subsequent analyses. Sediment water content was determined by drying at 105°C for 24 h.

**Chemical preparation and spiking**

Analytical standards of endosulfan and lindane were obtained at certified purity of 95.2% and 99.9%, respectively. Stock solutions of endosulfan and lindane at 5,000µg/l and 4,000µg/l respectively were prepared in analytical reagent (A.R.) acetone (Lab-Scan Analytical Sciences, Ireland). Spiking solutions of endosulfan and lindane were prepared at 5 and 4 µg/l, respectively by dilution with filtered natural seawater. Stock solutions were stored at 4°C in volumetric flasks.

**Sediment spike**

Direct sediment spiking method was used in this study (Stemmer *et al.*, 1990). Ten kilogram of sediment weighed into pre-cleaned acrylic chambers with 50 l capacity and spiked with suitable volumes containing nominal concentrations of endosulfan. lindane and combination of endosulfan and lindane in acetone on wet weight basis. Separate chambers were maintained for different spike tests. Sediment samples were immediately spiked soon after taken out from the refrigerator. Spiked sediment was thoroughly mixed with a probe for 20 min and transferred into a glass chamber of 10 l capacity for aging. Glass chambers were covered with a plastic sheeting and alu-foil wrapper prior to keep under 4°C in order to minimize volatilization. All glass chambers were stored for 24 h before they were taken for elutriate preparation to satisfy the sufficient time to equilibrate endosulfan and lindane in the sediment matrix (Sharom *et al.*, 1980a; Anurakpongsatorn, 1998; Zhou, 2003).

**Water spike**

The concentrations of test solutions were determined as required for the toxicity tests for shrimp postlarvae *Penaeus monodon* Fabricius. Suitable volumes containing nominal concentrations of endosulfan, lindane and its combination were pipetted into test seawater of 16% salinity.

**Water quality measurements**

Daily measurement of pH (CONSORT C932 electrochemical analyzer), dissolved oxygen and temperature (METTLER TOLEDO MO128 Dissolved Oxygen Meter equipped with IP67 Probe) were recorded in each test chamber during 9:00 to 11:00 a.m. The actual salinity was measured using a portable refractometer (Model: NOW, Tokyo, Japan). Total organic carbon in elutriates were measured by the Total Organic Carbon Analyzer (Model TOC-VCSN, Shimadzu Corporation) equipped with non-dispersive infrared detector with standard TC catalyst in high temperature combustion.

**Sample extraction and analysis for pesticides**

Replicated chambers were pooled in spiked-water tests for pesticide extraction after termination of the toxicity experiment at 96 h. Elutriates and water samples were stored in amber color reagent bottles (pre-cleaned and rinsed with acetone) under 4°C and filtered (Whatman GF/C glass microfibre filters) before extraction. The extraction of endosulfan and lindane in samples were conducted according to the procedure of USEPA method 8081B (USEPA, 2000). The samples were analyzed in HP 5890 series II gas chromatograph equipped with an electron capture detector (GC-ECD), HP

Chemstation Integration Software, HP 7673 GC/SFC injector and auto sampler system and HP5 capillary column (30×0.32×0.25 μm film thickness). The GC operating conditions were as follows: the column was held initially at a temperature 130°C for 2 min., then increased at 3°C/min. to 250°C and finally held at 250°C for 20 min. to remove column contaminants. The temperature of injector and detector were maintained at 225°C and 310°C, respectively. Nitrogen was used as a carrier gas at the flow rate of 1.1 ml/min. in the column. Standard solutions were prepared by dissolving standard mixture of pesticides (Pesticide 8081 Standard Mix in hexane: toluene (50:50) purchased from Sigma-Aldrich, Germany within the range of 0.1-40 μg/l of each pesticide in n-hexane and the calibration curves were linear within this concentration range. Identification of compounds was deduced from their retention times, and quantification was based on comparison of peak area with responses from reference standards.

**Statistical analysis**

All the data collections were made in triplicate unless otherwise stated and the values obtained were statistically processed to obtain the mean and standard deviation (SD). The data were analyzed using SPSS (Version 11.5 for Windows) statistical software program. Means were tested for homogeneity of variances before the test. Pearson correlation and regression in Post Hoc tests were conducted for analyzing data trends and correlations where necessary. Mean differences were considered significant when $p<0.05$.

## RESULTS AND DISCUSSION

**Dissolution potential in sediment**

Analysis of elutriate revealed that in an average (n=2) only 0.143 μg/l of endosulfan out of 100 μg/kg added in sediment was realized in the solution. Results of dissolution potential of endosulfan and lindane by Chantaburi estuary sediment are shown in Figure 1. The data indicate that the dissolution of endosulfan into the elutriate fraction was as low as 0.08-0.51% (mean 0.23%) of the endosulfan added while lindane at 1.2-3.4% (mean 1.84%). The particle size distribution in Chantaburi estuary sediment was 59.5% clay, 30.3% silt and 10.2% sand indicating high potential of adsorption capacity (Parkpian *et al.*, 2001).

Figure 1 shows the relationship of dose dependent endosulfan and lindane dissolution in sediment which indicates gradual decrease of dissolution as the spiked concentration of sediment increased. The negative nonlinear power regression indicates a better relationship (Pearson correlation) for the above observation and the coefficients of determination ($R^2$) for endosulfan and lindane were 0.97 and 0.87 respectively. One possible

explanation for these results might be due to surface adsorption with organic carbon substrates at lower concentrations while at higher concentrations, compounds that have been retained by organic matter would create the appearance of increasing desorption hysteresis (Bowman and Sans, 1985). The results in other words corroborate with studies of Parkpian *et al.*, (2001) on mercury desorption from Bangpakong river sediments that high energy bonding sites in internal surfaces of clay and organic matter would minimize toxicant release in to the water column.

Even though the water solubility of lindane is 100 times higher than that of endosulfan, the estuary sediment, however, did desorb slightly over 1.6% of endosulfan. This situation is similar to 8 times more dissolution of lindane than endosulfan in the estuary sediment. Comparatively higher dissolution is not surprising due to higher water solubility of lindane in water. This again reflects higher sediment binding potential of lindane irrespective of its water solubility potential. Either sorbed lindane may be released readily back into solution or un-sorbed amounts of lindane trapped in interstitial water may have released when the sediment was re-suspended in seawater while in preparation for elutriate. But, other possibilities can exist that considerable fraction of spiked dose is dissipated by competitive fate processes, such as volatilization, degradation and adsorption to container walls, *etc.*. Ramamoorthy (1985) had confirmed that wet sediment spiked with 700 µg/kg of lindane resulted in maximum of 0.6 µg/l in water leaving maximum of 77 µg/kg in sediment after 14 days. He further confirmed that in a sediment-water-biota test system these values can reflect for more than 100-300 times higher concentration of lindane in sediment than those in water. A reduction of endosulfan by 50% from spiked sediment during 7-day aging has been reported by You *et al.* (2004) where they speculated that degradation or firm binding with sediment matrix have made it less extractable. The sediment characteristics with respect to organic matter content in the sampling station was reported to be 5.5% and can be considered as high (Parkpian *et al.*, 2001). Under this condition, we might expect high affinity for sorption and low potential of desorption of hydrophobic contaminants from the sediment such as lindane and some forms of endosulfan.

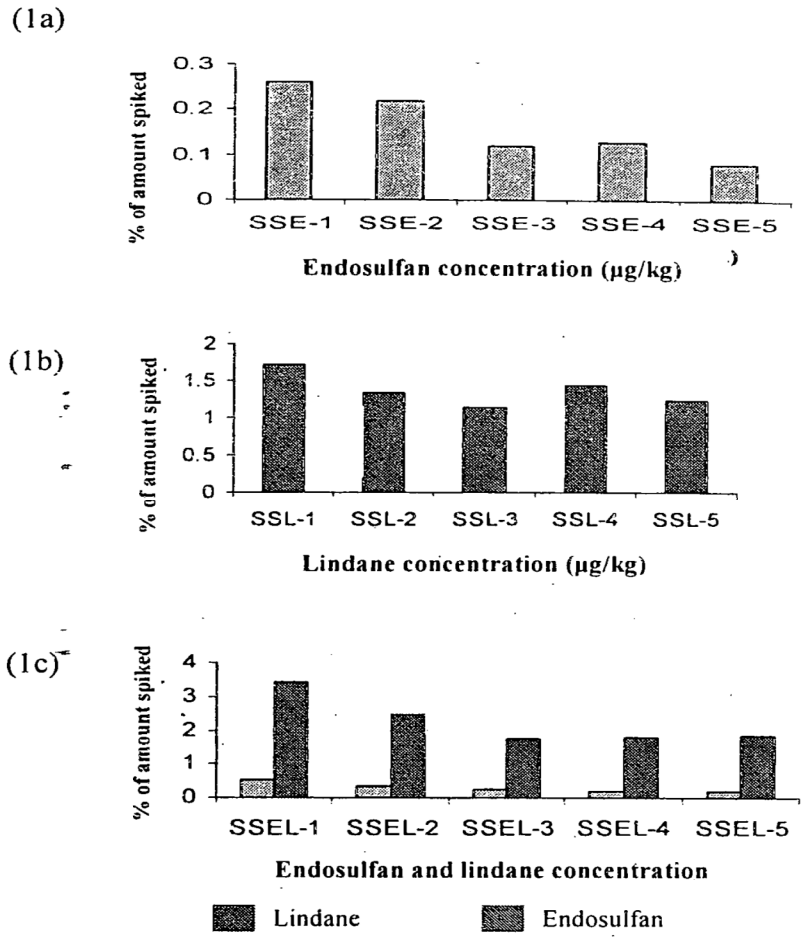


**Figure 1. Pesticide dissolution in sediment under different dose regimes (a) Endosulfan spiked-sediment elutriate (b) Lindane spiked-sediment elutriate (c) Endosulfan+Lindane spiked-sediment elutriate.**

Notes: SSE-1 = 50 µg/kg, SSE-2 = 100 µg/kg, SSE-3 = 400 µg/kg, SSE-4 = 1,000 µg/kg, SSE-5 = 2,000 µg/kg of endosulfan; SSL-1 = 25 µg/kg, SSL-2 = 50 µg/kg, SSL-3 = 200 µg/kg, SSL-4 = 500 µg/kg, SSL-5 = 1,000 µg/kg of lindane; SSEL-1 = 25+25 µg/kg, SSEL-2 = 50+50 µg/kg, SSEL-3 = 100+100 µg/kg, SSEL-4 = 400+150 µg/kg, SSEL-5 = 1,000+200µg/kg of endosulfan and lindane, respectively

Elutriate concentrations at 1:4 (v/v) sediment:water.

Endosulfan dissolution data based on α- and β-endosulfan isomers.

Data were not corrected for recovery; chromatographic recoveries [(surrogate standard measured/surrogate spiked) x 100%] ranged from 87-128% for endosulfan, 92-113% for lindane and 105-130% for endosulfan and lindane binary mixture.

## Remobilization of endosulfan

Figure 2 shows the remobilization of major forms of endosulfan from sediment into the aqueous phase during elutriate preparation. The endosulfan technical concentrate used to spike sediment had nearly 2:1 (w/v) ratio of α-endosulfan and β-endosulfan, respectively. Analysis of elutriate extracts in 12 samples for different concentrations of endosulfan spiked in sediment revealed that the average remobilization ratio was as same

as that of technical endosulfan; evidently 66.6% of α-endosulfan (n=12) and 33.4% of β-endosulfan (n=12) that complement the above statement. The above results are contradictory with the published information that according to Peterson and Batley (1993) mobility of β-endosulfan in sediment is slower than α-endosulfan because of less water solubility and correspondingly higher partition coefficient (Log $K_{ow}$). However, different fate processes acted upon α-endosulfan such as microbial hydrolysis (Sutherland *et al.*, 2000), chemical hydrolysis and volatilization (Leonard *et al.*, 2001), were faster than β-endosulfan indicating loss of α-endosulfan, lowering the true potential in the water column. It has been reported that during 6-11 weeks time α/β-endosulfan ratio was reduced from 2.2 to 1.3 from endosulfan spiked sediment under laboratory conditions (Leonard *et al.*, 2001). Endosulfan sulfate, the major degradation product of endosulfan, was analyzed to be in an average of 0.011 μg/l (n=12) in the elutriate extracts which is one order of magnitude less than for both α-endosulfan (>35 times) and β-endosulfan (>17 times). One of the aspects for low concentration of endosulfan sulfate would be low degradation in sediment under anaerobic conditions (Peterson and Batley, 1993) and poor release into the water column. It is expected that most of the β-endosulfan and endosulfan sulfate in the elutriate matrices may be associated with suspended particles while leaving more water soluble α-endosulfan in the true water phase (Peterson and Batley, 1993). The results of this study confirm that after vigorous re-suspension event of sediment (=elutriate), α-endosulfan would be the dominant fraction followed by β-endosulfan and endosulfan sulfate in order to cause biological effects.

**Dissipation in the water column**

In contrast to elutriates, endosulfan compounds measured in water after 96 h spiking were biased towards β-endosulfan where 2:1 ratio of α-endosulfan:β-endosulfan in the technical concentrate originally applied were transformed into 1:1.2 and 1:3 of α and β-isomers, respectively in different test matrices. Both water matrices clearly showed an increase of β-endosulfan fraction in the solution during 96 h test period compared to α-endosulfan (Fig. 3). The estimated nonlinear correlation (power model) ($R^2$=0.99) between β-endosulfan concentrations (%) in two independent spiked-water tests was significant at $p<0.05$ which suggests that environmental variables did not drastically affect endosulfan degradation. Analysis for the main degradation product, endosulfan sulfate revealed that in the spiked-water matrix (Fig. 3b) there was a significant ($p<0.01$) correlation (Pearson correlation) between spiked total endosulfan and endosulfan sulfate and the corresponding nonlinear regression (power) coefficient of determination ($R^2$) was 0.97. One of the possible reasons for comparatively higher β-endosulfan in spiked-water test matrix (Fig. 3a) could be due to higher pH (7.53-7.63) compared to the binary mixture test matrix (7.26-7.33) (Fig. 3b).

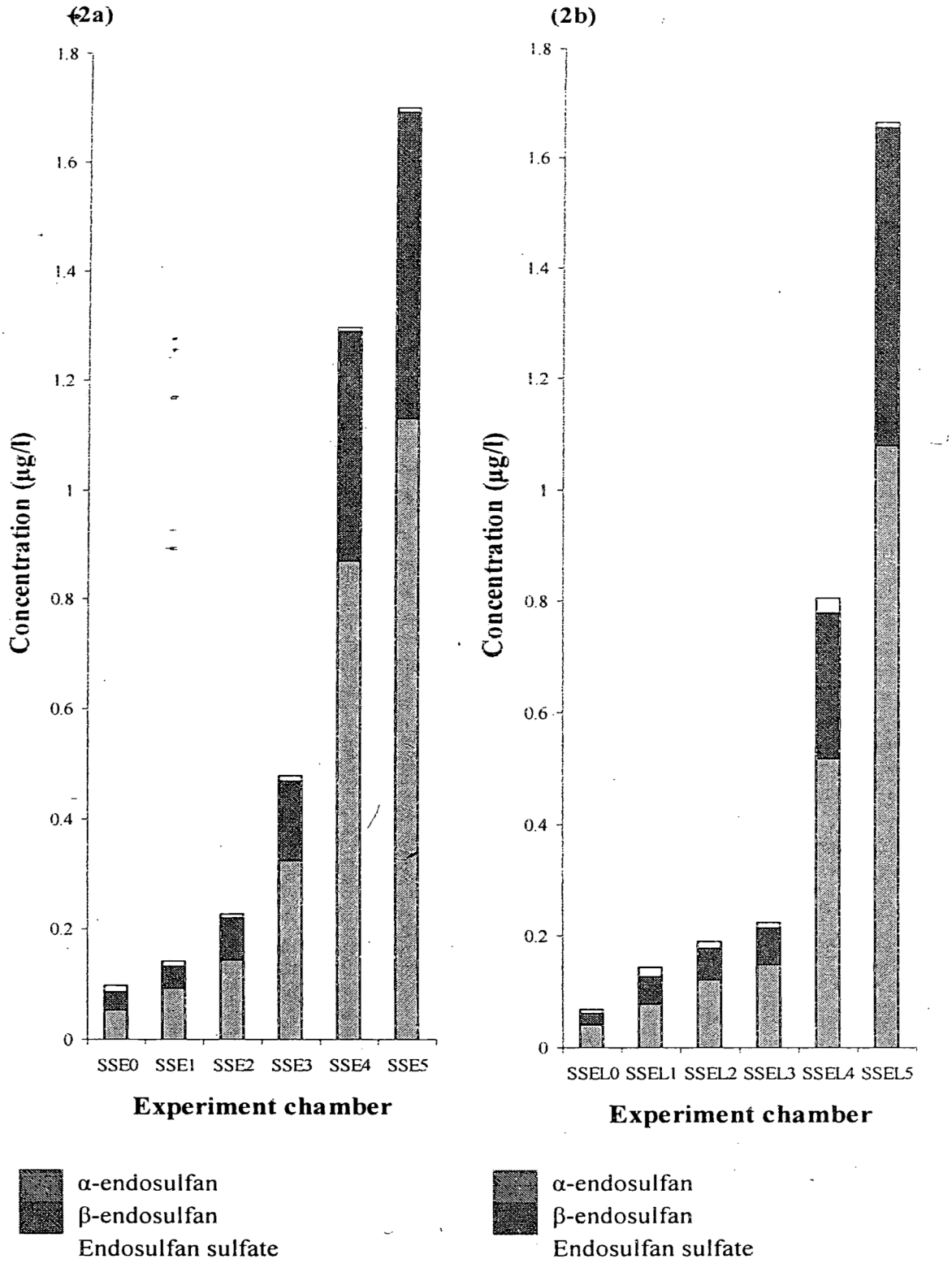


**Figure 2. Endosulfan concentration (including the main degradation product) in elutriate test matrices under different dose regimes (a) Endosulfan spiked-sediment elutriate (b) Endosulfan+Lindane spiked-sediment elutriate. SSE-0 = Un-spiked (control) SSE-1 = 50 µg/kg SSE-2 = 100 µg/kg SSE-3 = 400 µg/kg SSE-4 = 1,000 µg/kg SSE-5 = 2,000 µg/kg of endosulfan; SSEL-0 = Un-spiked (control) SSEL-1 = 25 µg/kg SSEL-2 = 50 µg/kg SSEL-3 = 100 µg/kg SSEL-4 = 400 µg/kg SSEL-5 = 1,000 µg/kg of endosulfan.**

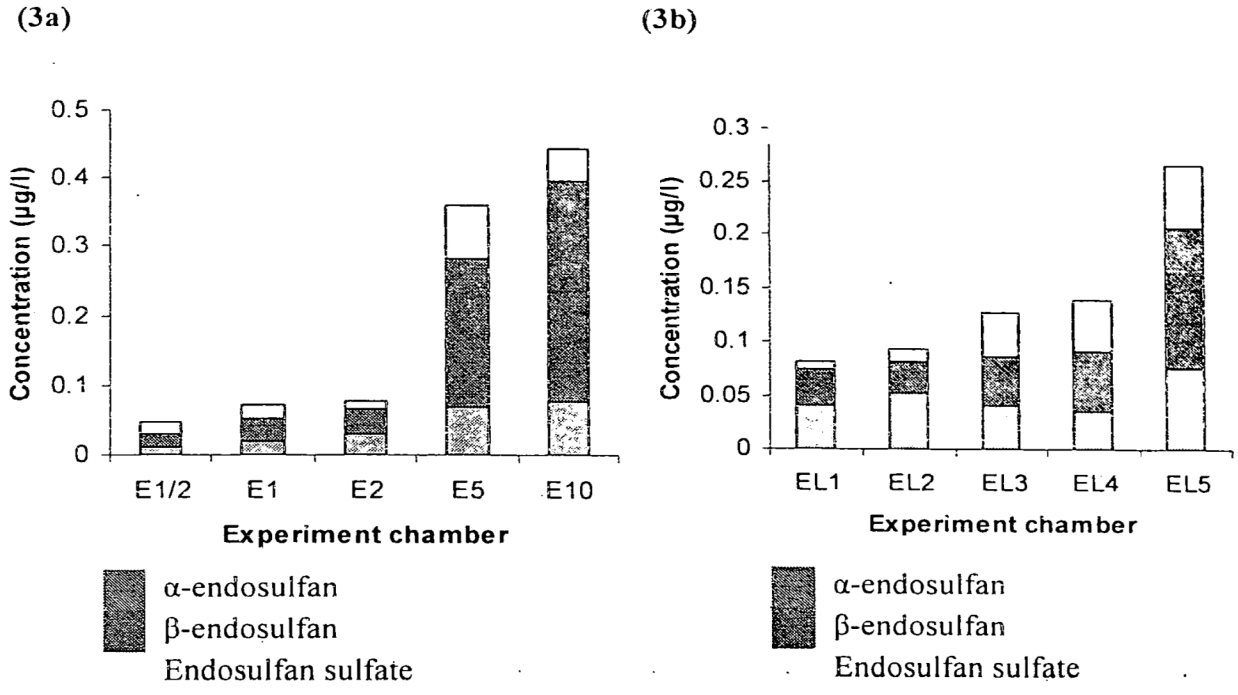


**Figure 3. Endosulfan concentration (including the main degradation product) in spiked-water test matrices under different dose regimes (a) Endosulfan spiked-water test matrix (b) Endosulfan+Lindane spiked-water test matrix. E1/2 = 0.5 µg/l spike E1 = 1.0 µg/l spike E2 = 2.0 µg/l spike E5 = 5.0 µg/l spike E10 = 10.0 µg/l spike; EL1 = 0.3 µg/l spike EL2 = 0.75 µg/l spike EL3 = 1.5 µg/l spike EL4 = 3.0 µg/l spike EL5 = 6.0 µg/l of endosulfan.**

Data were not corrected for recovery; Chromatographic recoveries [(surrogate standard measured/surrogate spiked) x 100%] ranged from 83-117% for endosulfan and 121-137% for endosulfan and lindane binary mixture.

Minimum method detection limit = 0.1 µg/l; Minimum matrix detection limit = 0.1 ng/l.

A significant loss of endosulfan and lindane in water test matrices was observed during 96 h. The mean total endosulfan remained at the 96 h was 7.52%. Some of the possible explanations for this finding could be due to possible loss of toxicants through volatilization and/or adsorption to the exposure vessels. Broomhall and Shine (2003) suggested that the decline in the nominal value of endosulfan concentration in a static non-renewal test over four days was due to adsorption to the glass vessels. In contrast to the loss of 20% endosulfan between nominal and measured concentrations during 96 h period (Broomhall and Shine, 2003), the degree of depletion was as high as 97% in some exposure vessels in this experiment (mean = 93.3%). Therefore, it is reasonable to assume that drastic losses observed in endosulfan may have occurred quite instantly, probably within few hours of spiking.

In contrast, the loss of lindane was lower than that of endosulfan where with a recorded maximum of 91% (mean = 81.9%), shows typically the persistent effect of lindane over endosulfan. Degradation of lindane in natural water has also been influenced by microbial action where

Sharom *et al.*, (1980b) demonstrated that more than 70% of the originally applied lindane can be diminished during 16 weeks.

From a comparison of chemical dissipation trends in test solutions over 96 h, it appears that the removal of carcass remains out of the test solutions may have affected the chemical existence in the test medium. The highest dissipation observed in high concentration test media may be due in part to the absorption by shrimp which was removed with the carcass when they were taken out of the test medium.

## CONCLUSIONS

The average dissolution potential was as low as 0.23% for endosulfan and 1.84% for lindane indicating strong affinity for hydrophobic contaminants in the Chantaburi estuary sediment. The relationship of dose dependent endosulfan and lindane dissolution in sediment indicates gradual decrease of dissolution with the increase of the spiked concentration of sediment. The negative non-linear power regression indicates a better relationship for the above observation. The coefficients of determination ($R^2$) for endosulfan and lindane were 0.97 and 0.87, respectively. This indicates that endosulfan and lindane dissolution into the water column from Chantaburi estuary sediment is rather conserved. The results confirmed that after vigorous resuspension event of sediment, α-endosulfan would be the dominant fraction followed by β-endosulfan and endosulfan sulfate in order to cause biological effects. However, the above observation masks the possibility of latent biological effects caused by some forms of endosulfan adsorbed onto colloidal particles. It is proposed that beyond speculations the exact mode of mechanism of adsorption-desorption should be experimented to assess the relative contribution of toxicants under controlled environments. In contrast to elutriates, β-endosulfan was the dominant form of compound in water after 96 h. Although there was no exact mechanism deduced for drastic depletion of endosulfan and lindane from test medium over relatively short duration of time (*i.e.*, 96 h), competing fate processes such as volatilization and sorption to surfaces of the container walls in addition to sorption to test organisms have depleted compounds from the water column. This study highlights the fact that test matrix components ultimately control the availability of a chemical in an aquatic system. Toxicity experiments designing in aqueous test matrices should consider chemical fate processes in determining exposure concentrations of the test chemical and interpreting data of test results.

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