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Distribution and Dissipation of Endosulfan and Related Cyclodienes in Sterile Aqueous Systems: Implications for Studies on Biodegradation

Turlough F. Guerin and Ivan R. Kennedy*

Department of Agricultural Chemistry, The University of Sydney, Sydney, NSW 2006, Australia

Endosulfans I (α) and II (β) dissipated in simple aqueous media in glass incubation vessels sealed with Teflon show apparent half-lives of 151 and 88 days at 30 °C, respectively. These values are significantly greater than others reported in the literature, because of precautions taken to prevent volatilization and biodegradation. Endosulfan sulfate, the major oxidation product of endosulfan, is much more persistent than either of the parent isomers under the same incubation conditions. A study of the distribution of the cyclodienes endosulfans I and II, aldrin, dieldrin, and endosulfan sulfate in a sterile aqueous microbial broth showed that these compounds were distributed to the glass/medium interface. The importance of the Henry's law constant and fugacity in the nonbiological loss of these compounds and precautions that need to be taken in studies of microbial degradation are discussed.

INTRODUCTION

The cyclodiene insecticides are of considerable significance to Australian agriculture and the environment. Although aldrin and dieldrin are no longer permitted for general use, dieldrin, in particular, is present in many waterways and cropping soils (Ang et al., 1989; Gilbert et al., 1990; McDougall et al., 1987). If pastures are grown on these soils and grazed, then livestock may become contaminated (Gilbert and Lewis, 1982). Endosulfan, on the other hand, does not bioaccumulate in mammals. It is currently registered for the control of insects in a range of crops, particularly cotton and canola, and has a vital role in the Pyrethroid Resistance Strategy which is currently proving successful in Australia for cotton production (Forrester, 1989).

Endosulfan is commonly classified as a chlorinated hydrocarbon of the cyclodiene group, on the basis of its chemical structure. Its physical, chemical, and physiological properties, however, are markedly different from those of aldrin and dieldrin (Maier-Bode, 1968). Endosulfan is readily metabolized in nontarget organisms, and this property arises from its relatively reactive sulfite group (Van Woerden, 1963). Endosulfan is considered to have a low level of persistence in the environment and a low toxicity to most higher organisms with the exception of fish, which are extremely sensitive to it (Goebel et al., 1982).

There are a number of papers describing the conditions favoring the chemical modification of endosulfan, and these have been reviewed by Goebel et al. (1982). There are, however, relatively few studies describing the fate of endosulfan in simple, well-defined, nonbiological aqueous systems (Greve, 1971; Martens, 1976; Miles and Moy, 1978; Singh et al., 1991). Thus, there is a need for a greater understanding of the fate of endosulfan in aqueous media, particularly in relation to biodegradation studies, where it is important to distinguish between the mechanisms of nonbiological and biological loss. It has not been conclusively determined to what extent losses of endosulfan result from degradation as against dissipation by other means, such as volatilization, in aqueous incubation systems. The primary aim of this study is to describe the nonbiological dissipation of endosulfan in aqueous media under well-defined conditions, preparatory to a study of the microbial degradation of endosulfan. The ways in which nonbiological losses of endosulfan can be reduced in biodegradation systems have been addressed. Consideration has also been given to the reasons for the observed physical behavior of the pesticides in these experiments.

* Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Chemicals. Endosulfan and its metabolites were a gift from Dr. Klaus Stumpf, Hoechst AG, Frankfurt am Main, FRG, and Hoechst Limited Australia. Aldrin and dieldrin were provided by Shell Chemicals, Australia. *cis*- and *trans*-aldrin diol were kindly provided by Dr. David H. Hutson, Shell Chemicals Research, Sittingbourne, Kent, U.K. Hexane (Nanograde) and methanol (ChromAR) were purchased from Mallinckrodt Chemicals. Tween 80 (polyoxyethylene sorbitan monooleate) was purchased from Sigma Chemical Co. All other chemicals used were of analytical grade.

Incubation Conditions. Pesticides and their metabolites (100–500 ppm in methanol) were added to either Nanopure-filtered, sterile, distilled, and deionized water or sterile 100 mM potassium phosphate buffered soil extract yeast mannitol medium in 4-mL Wheaton vials, to give final amounts of 1–5 μg mL^{-1}. The level of added pesticide exceeded the limits of the solubility for these compounds in the aqueous phase. The pesticides were delivered in 10 μL of methanol. Duplicate vials were made of unsilanized borosilicate glass and prepared by washing in concentrated sulfuric acid followed by rinsing in acetone prior to use. The medium contained K_2HPO_4 (anhydrous) 6.79 g L^{-1}, KH_2PO_4 (anhydrous) 8.30 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.05 g L^{-1}, $FeSO_4 \cdot 7H_2O$ 0.01 g L^{-1}, yeast extract (Difco) 2.00 g L^{-1}, mannitol (Sigma) 2.00 g L^{-1}, sucrose (Pronalysis) 2.00 g L^{-1}, and soil extract 100 mL L^{-1} (Allen, 1957). Vessels were sealed with either poly(tetrafluoroethylene) (PTFE)-lined silicone rubber seals (Wheaton, Millville, NJ, supplied by Edwards Instrument Co., Narellan, Australia) or non-PTFE-lined butyl rubber stoppers. Duplicate vessels were kept at 30.0 ± 0.5 °C, in an air flow controlled incubator for 30 days. No further attempt was made to artificially aerate the incubation flasks during the course of the experiment, and sterile air at atmospheric pressure was used as the gas atmosphere, from a laminar flow cabinet. To maintain the incubations sterile throughout the experiment, sodium azide was added at 0.5% (w/v). The pH of all the media was adjusted to 7.00 ± 0.05 with KOH or HCl prior to dispensing into the incubation vessels.

Extraction and Recovery of Parent Pesticides and Metabolites. Duplicate incubation vessels containing the aqueous media (500–1000 μL) had their contents quantitatively transferred into the reservoir of a 10-mL liquid/liquid partitioning device

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(Mixxor by Genex) and were extracted according to the method previously developed (Guerin et al., 1992). Samples were taken on days 0, 2, 4, 6, 8, 15, and 30. A total solvent volume of 10 mL of hexane/acetone/methanol/medium (15:5:2:2) was also added to the Mixxor reservoirs. The piston of the Mixxor was moved 60 times in its reservoir to partition the pesticides into the solvent phase. After the phases were allowed to separate (approximately 1 min), the solvent layer was decanted off the aqueous phase directly from the Mixxors into volumetric flasks and the total volumes were made to either 10.0 or 25.0 mL with hexane. Subsamples were dried with anhydrous sodium sulfate prior to analysis. Cleanup in the experiment was not necessary as there were no interfering peaks recorded on the chromatograms. Recoveries (percent) of the parent compounds and their standard deviations at the 1 ppm level were as follows; aldrin 85 ± 2.9; dieldrin, 88.5 ± 4; endosulfan I, 89.4 ± 4.1; and endosulfan II, 82.7 ± 4.1. The recovery of endosulfan sulfate at the 5 ppm level was 83.9 ± 6.6. Recovery of the aldrin diols and endosulfan diol was 70 ± 7.1 at the 0.5–1.0 ppm level of spiking. Residue levels were plotted as log $100 \times C/C_0$, where C is the concentration at time (t) and C_0 is the concentration at t_0. The values for C_0 were 1 ppm for aldrin, dieldrin, and endosulfans I and II and 5 ppm for endosulfan sulfate. An assumption was made that the degradation and disappearance of the compounds followed first-order kinetics at constant pH. The equation used to plot the degradation in this study was that used by Cotham and Bidleman (1989). Half-lives were determined by plotting a line of best fit through the data points calculated using the above equation.

Gas Chromatographic Analysis. The analytical conditions were as previously described (Guerin et al., 1992). Extracts were analyzed on a Shimadzu GC 8A ^{63}Ni ECD gas chromatograph. Columns used were an OV-210 (5%):SE-30 (5%) (7:3) on Chromosorb W-HP, 80/100 mesh, and an SE-30 (5%):DC-200 (5%) (3:1) on Gaschrom Q 80/100 mesh packed into 3 m × 2.0 mm custom-made unsilanized borosilicate glass columns. The former column was used for confirmation of pesticides and their metabolites only. The N_2 gas flow rates on both columns were adjusted to 25 mL min^{-1}. The column and injector/detector temperatures were set at 210 and 290 °C, respectively. Chromatograms were recorded on a Shimadzu CR-3A integrator.

Metabolite Identification. Metabolites were identified by comparing retention times of metabolite standards, prepared in hexane, with the degradation product peaks appearing on the treatment chromatograms. Peak identity was confirmed by consistent retention time and coelution with standards on two different chromatographic columns under the conditions previously described (Guerin et al., 1992). All of the compounds analyzed were measured at concentrations within their linear range on the ECD gas chromatograph.

Determination of Pesticide Distribution. Three types of media were employed in determining the distribution of pesticides in the incubation vessels, which were duplicate PTFE-sealed Wheaton vials (4 mL). These media were as follows: (1) Nanopure-filtered, sterile, distilled, and deionized water; (2) growth medium with pesticide (1–5 μg mL^{-1} added in 10 μL of methanol); and (3) growth medium and pesticide and 0.1% Tween 80. In all three treatments, half the total amount of medium originally added was carefully subsampled by removing the liquid with a narrow-bore pipet from the bulk after 4 h of incubation at 30 °C. Losses of pesticide during this equilibrium period were found to be negligible, so there was not measurable degradation or dissipation from the vessels over the 4-h period. The subsampled fractions were termed the medium bulk. The remaining medium, which contained the medium at the interfaces, i.e., on the glass walls and on the liquid/air interface, was termed the interface medium, and all of these remaining residues were collected by rinsing the incubation vessels into the extraction chambers of the Mixxors. Both the subsamples and rinsed fractions were extracted individually and analyzed for the cyclodiene pesticides, using the method described under Gas Chromatographic Analysis and further described in Guerin et al. (1992).

Surface microlayer subsamples in triplicate were also taken after 4 h (30 °C) from vessels set aside especially to determine whether there was any difference in pesticide concentration at the liquid/air interface. This was performed by withdrawing a volume of 100 μL from the surface, an amount equivalent to the top 0.9 mm of the medium, using a pipet. The pipet was rinsed with solvent and the medium extracted as described under Extraction and Recovery of Parent Pesticides and Metabolites. An equivalent volume removed from the medium bulk was also analyzed for pesticides.

RESULTS AND DISCUSSION

Losses of Aldrin and Dieldrin. Initial experiments were performed to indicate how much pesticide loss could occur by escape from the aqueous phase from unsealed flasks or flasks sealed with butyl rubber during 4 weeks of incubation at 30 °C. These are the conditions under which studies of microbial degradation of pesticides are often performed (Wallnofer and Engelhardt, 1990; MacRae, 1989, and references cited therein). In aerobic microbial degradation studies, cotton wool has often been employed as a stopper to keep the flask contents sterile or prevent contamination by other microorganisms. In anaerobic studies, butyl rubber is often used to seal the flasks to prevent contamination by oxygen. In these initial experiments, there were high rates of loss of aldrin and dieldrin from both unsealed incubation vessels and flasks sealed with butyl rubber (Figure 1A,B). In the butyl rubber sealed vessels, aldrin and dieldrin had largely disappeared at the 30th day, with apparent half-lives of 8.3 and 16 days, respectively. These results were similar to the values of 5.8 and 17 days for half-lives in the open flasks. The difference between the two systems was that the initial rate of dieldrin disappearance in the unsealed vessels was slightly higher than in the butyl rubber sealed vessels. In contrast, when aldrin and dieldrin were incubated in similar sterile media, but with PTFE-lined silicone rubber seals, there was no apparent loss (Figure 1C,D).

The pattern of loss for aldrin from butyl rubber sealed flask and that of dieldrin from unsealed flasks appeared to be biphasic, i.e., high early in the experiment, decreasing to a much lower rate of loss at 30 days. A possible reason for this non-first-order loss could have been that a large proportion of pesticide was concentrated at the liquid–air interface (surface microlayer) and, as a consequence, is volatilized early in the experiment. After this surface quantity had all volatilized, further losses must come from the solution bulk through the process of diffusion. Another possible reason for this biphasic loss may be because of a rim effect, where the more rapid diffusion occurs at high adsorptions (i.e., at concentrations higher than the compound's solid-phase solubility early in the experiment) across the glass to the surface. The latter explanation best explains the pattern in this experiment, because there was no detectable concentration of insecticides at the liquid–air interface (see Distribution of Pesticides in the Aqueous Incubation System).

The inclusion of the very stable cyclodienes aldrin and dieldrin in this study provided internal controls that indicated disappearance predominantly from physical losses, thereby providing the maximum limits of these processes in the system. The experimental conditions were too mild and the incubation period was too short to allow any chemical degradation of these compounds (Worthing and Walker, 1987; Singh et al., 1991). The persistence of aldrin and dieldrin in these incubations therefore represents the maximum limits for either slow volatilization or other processes such as irreversible binding to glass. Thus, any differences between the persistence of the internal controls and that of endosulfan represent the actual disappearance owing to chemical reaction. The very slow rate of disappearance of aldrin and dieldrin in the PTFE-

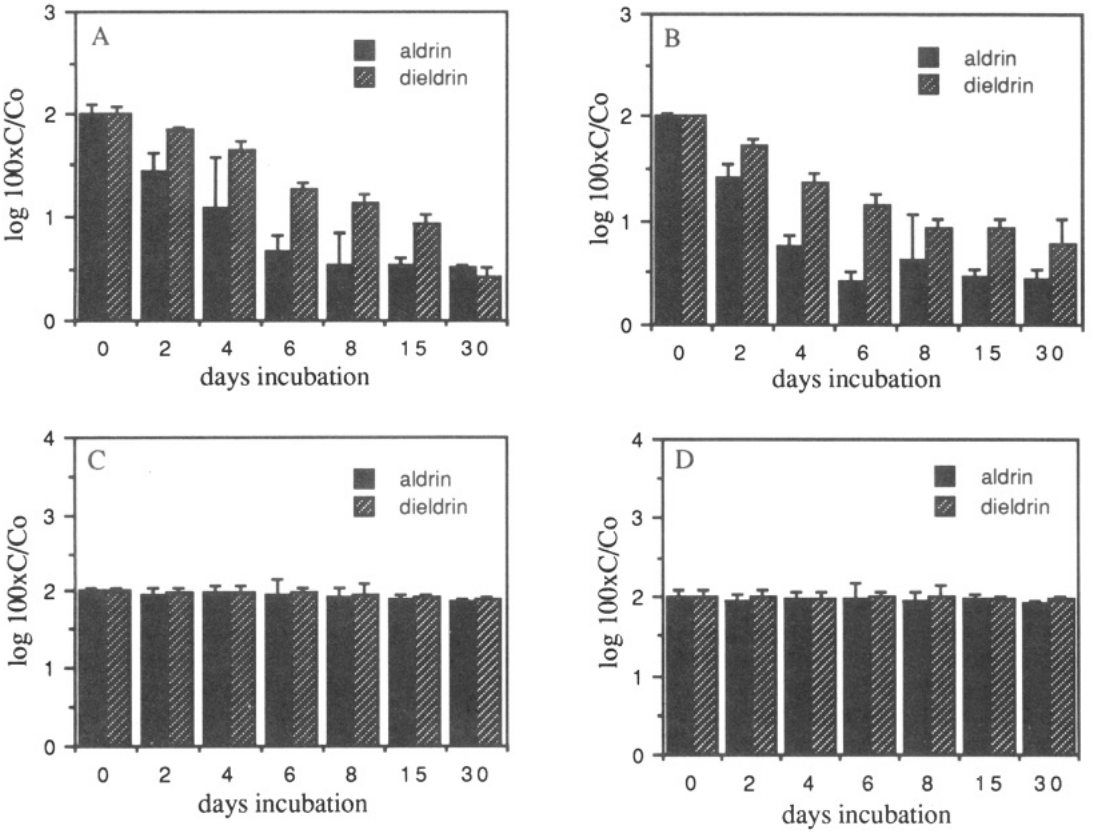

Figure 1. Dissipation of aldrin and dieldrin from (A) growth medium in butyl rubber sealed vessels, (B) growth medium in unsealed vessels, (C) growth medium in PTFE-sealed vessels, and (D) water in PTFE-sealed vessels.

sealed vessels in both water and growth medium confirmed that the system was well sealed.

Losses of Endosulfan. After the losses of the cyclodienes from the incubation vessels had been minimized, the persistence of endosulfan was examined. In both the butyl rubber sealed and the unsealed vessels, the endosulfan isomers were lost at high rates. After 30 days of incubation, the calculated half-lives of endosulfans I and II in the butyl rubber sealed flasks were 26 and 42 days, respectively. When endosulfans I and II were incubated in unsealed vessels, the half-lives were 19 and 75 days, respectively (Figure 2A,B).

When the vessels were sealed with PTFE, rates of disappearance for both isomers were much lower in both water and microbial growth medium (Figure 2C,D). In the growth medium, this corresponded to half-lives of 151 and 88 days, respectively, for endosulfans I and II. In water, the half-lives were 157 and 90 days for endosulfans I and II, respectively. The PTFE treatment presumably reduced the volatilization of the parent compound from the flasks. Endosulfan I, however, dissipated at a slower rate than endosulfan II in both media under these conditions.

The higher rate of dissipation of endosulfan II from both water and microbial medium in PTFE-sealed vessels reinforces the conclusion that endosulfan II is the more chemically labile of the two isomers (Goebel et al., 1982). Given the relative chemical inertness of the PTFE-sealed systems and that traces of endosulfan diol were detected in the same system, it is reasonable to conclude that both endosulfan isomers were chemically degraded in the aqueous incubations. There was very little difference in the degradation rates of either isomer between the water and the microbial growth medium incubations. This indicates that the soil, peptone, or yeast extract and inorganic minerals had little effect on the persistence of the isomers. Van Woerden (1963) reports that the hydrolysis of organic sulfites is not affected by the presence of metal ions when dissolved in aqueous solution. One exception has been reported with *o*-phenylene sulfite, which can be hydrolyzed at a faster rate in the presence of bisulfite ions (Van Woerden, 1963).

In previous studies a general trend has been that endosulfan II disappears at a faster rate than endosulfan I, and these have been summarized in Table I. Miles and Moy (1979) reported that under aerobic conditions at a lower temperature of 22 °C the half-lives of endosulfans I and II in a potassium phosphate buffered, minimal salts medium (pH 6.5) were 88 and 40 days, respectively. In their paper no mention was made on how the vessels were sealed. Cotham and Bidleman (1989) described the degradation of endosulfan in seawater and seabed sediments incubated in flasks sealed with polyurethane. The half-lives of endosulfans I and II were 4.9 and 2.2 days, respectively, in nonsterile seawater (pH 8.0). These incubations were carried out aerobically and at 20 °C under laboratory lighting. In another study, incubations in lake water (Greve and Wit, 1971) showed that the half-life of endosulfan I was 35 days at pH 7 and 105 days at pH 5.5. It was shown in the same study that when iron hydroxide gel is mixed with water, the rate of hydrolysis is considerably accelerated. The data in Table I show that both isomers of endosulfan are much more chemically stable than previous papers would suggest. The shorter half-lives reported in previous studies are presumably a result of either nonsterile conditions or volatilization.

When the different investigator's half-lives are compared, the role of pH is important. This is because the endosulfan isomers are susceptible to alkaline hydrolysis (Goebel et al., 1982). Therefore, rates of hydrolysis at pH 8 will be 10 times faster than the rates at pH 7. Some differences in the half-lives reported may be due to

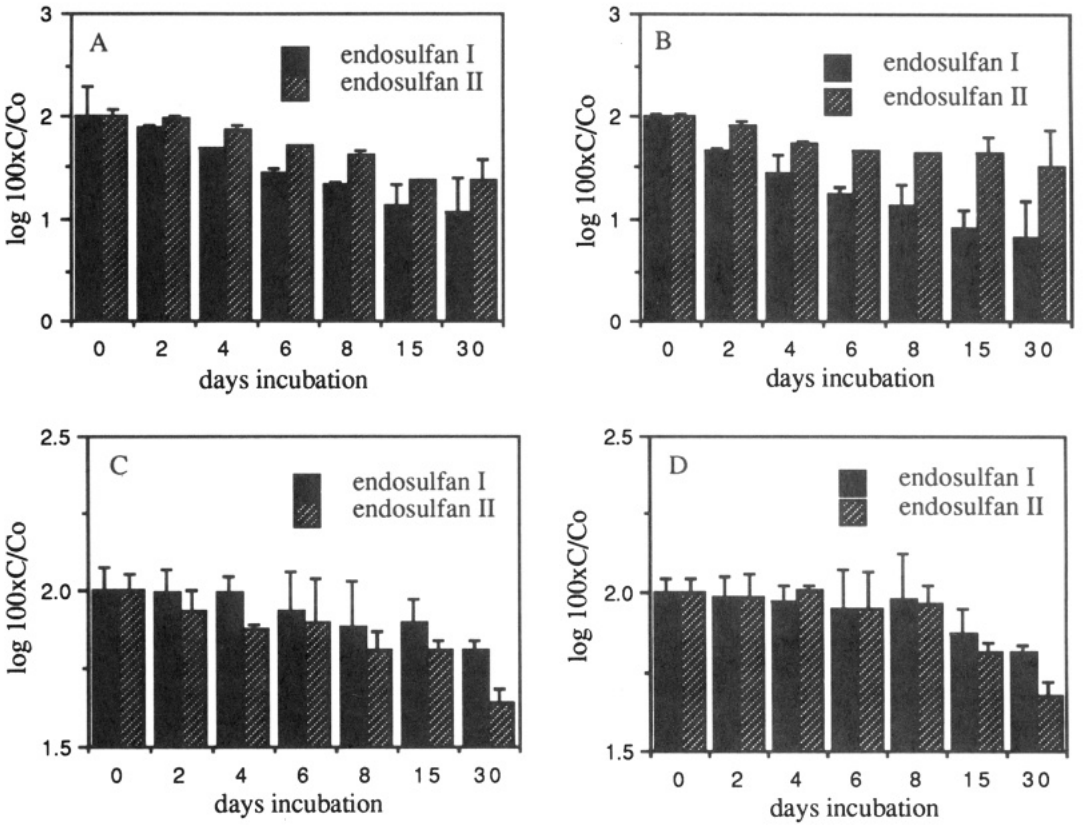

Figure 2. Dissipation of endosulfan isomers from (A) growth medium in butyl rubber sealed vessels, (B) growth medium in unsealed vessels, (C) water in PTFE-sealed vessels, and (D) growth medium in PTFE-sealed vessels.

Table I. Comparison of the Current Study with Reported Half-Lives of Endosulfan in Sterile Aqueous Media[a]

type of aqueous medium	vessel type and seal	pH	temp, °C	subsampling[b]	apparent half-life, days	
					endosulfan I	endosulfan II
lake water[c]	–	7	–	–	35	–
river water[d]	–	7.3–8.0	room temp	–	4.0[e]	–
phosphate medium[f]	glass and cotton wool	7.0	27	–	5.5[e,g]	–
phosphate medium[h]	–	6.5	22	yes	88	40
seawater[i]	glass and polyurethane	8.0	20	no	4.9	2.2
distilled water[j]	glass vessel	6–7	25	–	5.2	4.2
river water[k]	glass and foil	7.2	room temp	no	49	22
phosphate–citrate KCl buffer[l]	–	7.0	30	yes	27.5	23.5
phosphate medium,[m] current study	glass and Teflon	7.0	30	no	151	88
distilled water, current study	glass and Teflon	7.0	30	no	157	90

[a] Data from the literature were chosen from experiments conducted with simple aqeous media at pH 7 ± 1. [b] Indicates whether an aliquot of media was removed from a larger total volume at each sampling time. [c] Greve and Wit (1971). [d] Eichelberger and Lichtenberg (1971). [e] Assuming first-order kinetics. [f] Martens (1976). [g] Formation of endosulfan diol was used as an indicator of loss of endosulfan. [h] Miles and Moy (1978). [i] Cotham and Bidleman (1989). [j] Meenatchi-Sunderam (1990). [k] Peterson and Batley (1991). [l] Singh et al. (1991). [m] Allen (1957). A dash indicates there were no specific information reported.

differences in temperature, which may also affect the hydrolysis rates of pesticides. The values from the literature used in Table I were those in which the studies were conducted within the pH range 6–8 and the temperature range 20–30 °C. Thus, not all of the studies reported in Table I are directly comparable. However, the specific values for these parameters are given, where reported in the literature, in Table II.

Losses of Endosulfan Sulfate. In all of the experiments carried out, endosulfan sulfate was very stable and considerably more persistent than the parent isomers (Figure 3A,B). The apparent half-life of endosulfan sulfate in the sterile aerobic vessels was 184 days when sealed with PTFE and 96 days in the unsealed vessels. Its persistence was only slightly higher in the vessel sealed with butyl rubber, compared to that in the unsealed vessels. Miles and Moy (1979) have also reported on the persistence of endosulfan sulfate in aqueous media and give a value for its half-life, under the previously described conditions, as greater than 140 days. The persistence of endosulfan sulfate in the aqueous systems studied in the current paper indicates that this endosulfan transformation product may remain in some water environments longer than the parent isomers.

Role of Volatilization in Pesticide Disappearance. From the increased losses in the open-vessel incubations, it is evident that the endosulfan dissipation in these experiments was primarily owing to volatilization. Endosulfan I disappeared at a much faster rate than endosulfan II, as expected, since the former is more volatile and less water soluble. The relatively high volatilization rate of endosulfan I has previously been reported from solid surfaces as well as aqueous systems (Beard and Ware, 1969; Singh et al., 1991). The rates of volatilization of the endosulfan isomers in the open-vessel experiments were similar to those with the butyl rubber sealed experiments,

Table II. Liquid-Phase Physical Properties of Endosulfan and a Comparison with Related Compounds

compound	water solubility ppm	water solubility mol m^3	log K_{ow}[a]	vp,[b] Pa	H,[c] Pa mol^{-1} m^3
aldrin	0.37 ± 0.49[d]	1.01×10^{-3}	3	0.005 ± 0.004	4.95
dieldrin	6.0 ± 0.67[e]	1.58×10^{-2}	3.7	0.0083 ± 0.006	0.53
endosulfan I	2.29 ± 1.32[f]	5.63×10^{-3}	3.6	0.006	1.07
endosulfan II	31.1 ± 7.58[g]	7.6×10^{-2}	–	0.003	0.04
endosulfan sulfate[h]	18.14	4.39×10^{-2}	–	–	–
endosulfan diol[h]	300.0	8.31×10^{-1}	–	–	–
endosulfan ether[h]	1392.8	4.06	–	–	–

[a] Octanol/water coefficient at 20 °C in the aqueous phase from Suntio et al. (1988). [b] Vapor pressure at 20 °C from Suntio et al. (1988) and Cotham and Bidleman (1989). [c] Henry's constant calculated from the vapor pressure divided by water solubility. [d] At 20–25 °C from Park and Bruce (1968), Weil et al. (1974), Biggar and Riggs (1974), and Kenaga (1980a,b). [e] 25 °C from Biggar and Riggs (1974), Eye (1968), Gunther et al. (1968), Weil et al. (1974). [f] 22–25 °C from Phillips (1975), Weil et al. (1974), and Worthing and Walker (1987). [g] 20–25 °C from Phillips (1975), Weil et al. (1974), Worthing (1987), Bowman and Sans (1983a). [h] 20 °C from Hoechst (1986). A dash indicates that the authors could not obtain data for those compounds.

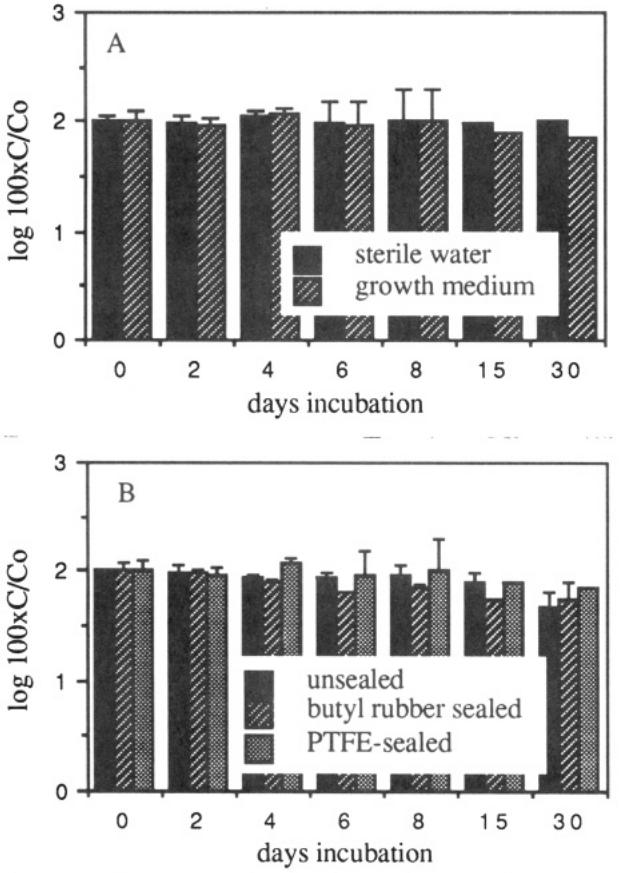

Figure 3. Dissipation of endosulfan sulfate in (A) water and growth medium in PTFE-sealed vessels and (B) growth medium with various types of sealing.

indicating that volatilization of these compounds from the aqueous media is not reduced by the presence of the butyl rubber seal. Aldrin and dieldrin were also lost at fast rates in similar incubations, confirming that either solubility in, or volatilization through, the butyl rubber seals was the major cause of loss. No hydrolysis products of endosulfan, endosulfan sulfate, aldrin, or dieldrin were detected in hexane/acetone extracts from the open or butyl rubber sealed treatments, thereby providing evidence that the dissipation of all compounds in the non-PTFE-sealed flasks was primarily a result of volatilization and not chemical degradation.

Volatilization from uninoculated controls in aerobic microbial degradation studies is likely to be a significant factor in overall pesticide disappearance in unsealed systems, particularly with pesticides such as endosulfan. In the recent study by Cotham and Bidleman (1989), 30% of applied endosulfan I was reported to have volatilized from a sediment/seawater incubation system (sealed with polyurethane) during the first 4 days of the experiment. The current findings therefore confirm the necessity to seal such systems with an inert material. The high volatilization rate of endosulfan I, also illustrated in other studies (Beard and Ware, 1969; Singh et al., 1991), is due to its low water solubility and relatively high vapor pressure, or its high Henry's law constant. The ratio of liquid-phase vapor pressure and solubility, or solid-phase vapor pressure and solubility, provides a value for the Henry's law constant (Suntio et al., 1991; Gerritse et al., 1991). This relationship may be used to show the difference in the relative rates of volatilization of the parent endosulfan isomers (Cotham and Bidleman, 1989; Goebel et al., 1982, and references cited therein) and of the recalcitrant cyclodienes, aldrin and dieldrin (Table II).

In calculating the Henry's law constant, values for water solubility and vapor pressure must be for the same phase, i.e., both for the liquid phase or both for the solid phase. The values presented in Table II are for the liquid phase for each of the pesticides, which were converted from the properties of the solid compounds, reported throughout the literature, using eq 1 (Suntio et al., 1988). In eq 1, F

$$F = P_S/P_L = C_S/C_L = \exp[-0.023(T_M - 298)] \quad (1)$$

is the fugacity ratio of the compound under study, C_S is the solubility of the solid compound, and C_L is the solubility of the liquid compound. P_S is the vapor pressure of the compound as the solid phase, and P_L is the vapor pressure of the liquid compound. T_M is the melting point of the compound in Kelvin. The exponent in the equation takes into account the entropy of fusion of the solid compound, and since there are often no experimental data available, the value of −0.023 is generally adopted (Suntio et al., 1988).

In illustrating the importance of the Henry's law constant of a compound, it is convenient to introduce the concept of fugacity. The fugacity is the escaping tendency of a compound from a particular phase (eq 2). In eq 2, f

$$f = C/Z \quad (2)$$

if the fugacity (units of pressure Pa), C is the concentration (units of mol m^{-3}), and Z is the fugacity capacity (units of mol m^{-3} Pa^{-1}). Each phase has its own fugacity, and at equilibrium, compounds will accumulate in phases with the lowest fugacity, or highest Z values. So in water the fugacity capacity is the inverse of the compound's Henry's constant (H) (eq 3) (Connell, 1991; Barber and Davis, 1991).

$$Z = Z_{water} = 1/H \quad (3)$$

The calculated fugacities of the cyclodiene compounds under study are equivalent to their vapor pressure values

Table III. Percentage Distribution of Aldrin, Endosulfan Isomers, and Their Major Oxygenated Metabolites toward the Interfaces and Medium Bulk in Borosilicate Glass Vessels[a]

	growth medium only		growth medium + Tween 80		water + Tween 80		
compound	IM[b]	MB[c]	IM	MB	IM	MB	CV,[d] %
aldrin	39.6 ± 4.3	60.4 ± 6.5	2.8 ± 0.2	97.2 ± 7.7	6.7 ± 0.4	93.3 ± 5.3	8.2
dieldrin	51.3 ± 8.5	48.7 ± 8.1	5.9 ± 0.9	94.1 ± 14.1	6.9 ± 0.3	93.1 ± 3.9	11.9
endosulfan I	38.9 ± 0.9	61.1 ± 1.5	0.0 ± 0.03	100.0 ± 4.9	8.2 ± 0.4	91.8 ± 4.5	4.0
endosulfan II	49.8 ± 4.7	50.2 ± 4.7	0.4 ± 0.04	99.6 ± 9.8	3.8 ± 0.1	96.2 ± 3.5	5.8
endosulfan sulfate	45.2 ± 1.5	54.8 ± 1.8	2.5 ± 0.1	97.5 ± 4.2	2.9 ± 0.1	97.0 ± 3.9	3.9

[a] Conditions of the incubation are described under Materials and Methods. Values are in percentage of total recovered ± standard deviation determined from the duplicate extractions. [b] IM, interface medium, includes both the liquid–glass and liquid–air interfaces of the closed system. [c] MB, medium bulk, subsampled from the total volume of medium. [d] Coefficient of variation determined from the average standard deviation for each of the compounds across all treatments.

in the same phase because the concentration (C) is equal to their water solubilities for the solid compounds. The solubility data used in Table II are those values reported from shake flask experiments conducted at 20–25 °C with analysis by ECD gas chromatography and converted to liquid-phase solubilities using eq 1, many of which were compiled from Shui et al. (1990) and references cited therein. The values for vapor pressure were selected from the data compiled by Suntio et al. (1988) and from Cotham and Bidleman (1989). These values were determined from gas chromatographic analyses where the retention times of the unknowns were compared to structurally related compounds with known and reliable vapor pressures, using nonpolar chromatography columns.

Some of the behavior observed in the butyl rubber sealed and unsealed vessels can be accounted for by differences in their calculated fugacities. The fastest rates of disappearance from both of these treatments was that of aldrin, which also had the greatest fugacity. Endosulfan I has a Henry's law constant approximately 27 times that of endosulfan II, which correlates well with the greater rate of disappearance from the non-PTFE-sealed vessels. The behavior of dieldrin, however, cannot be explained by its calculated fugacity alone, as this value is many times less than that of aldrin, yet its rate of disappearance was high. Direct measurements of the Henry's law constant for dieldrin have been reported (Suntio et al., 1988; Atkins and Eggleton, 1971; Slater and Spedding, 1981). An average value calculated from the literature is 3.4. This is a high value relative to those calculated indirectly in Table II. This may explain why dieldrin appears to be more volatile, in the current study, than expected from the calculated Henry's law constant.

Detection and Analysis of Potential Hydrolysis Products. Trace levels of the hydrolysis product, endosulfan diol, were detected in the incubations containing endosulfan initially. Data from the recovery experiments showed that this potential degradation product, when spiked into zero-time vessels, was being extracted, and its identification was confirmed using two different gas chromatographic conditions (Guerin et al., 1992). The highest levels of endosulfan diol were detected in the PTFE-sealed incubations. With endosulfan I, these levels were 0.08–0.1 ppm of diol after 30 days. This rate of diol formation correlates well for the calculated half-life of endosulfan I of approximately 150 days in the sterile media. Slightly higher levels of 0.1–0.15 of ppm endosulfan diol were detected in the endosulfan II incubations under the same conditions, consistent with its low stability. Only trace levels of endosulfan diol (<0.03 ppm) were detected in the endosulfan sulfate incubations and then only in PTFE-sealed incubations.

The potential hydrolysis products of dieldrin, *cis*- and *trans*-aldrin diol, were not detected in any of the treatment incubations containing dieldrin, although the underivatized standard compounds were chromatographed successfully under the conditions described for analyzing the parent compounds (Guerin et al., 1992). Given the highly recalcitrant nature of dieldrin, and the mild incubation conditions of water and growth medium, no hydrolysis products would be expected to form. Another recent paper confirms this stability of dieldrin in aqueous systems (Singh et al., 1991).

Distribution of Pesticides in the Aqueous Incubation System. Approximately 40–50% of the total amount of pesticides recovered from the growth medium (1–5 $\mu g\ mL^{-1}$ originally added) was found to be concentrated at the interfaces of the system (Table III). An attempt was made to subsample the liquid–air interface and determine pesticide concentration. However, there was no detectable difference between the surface sample and that in the bulk of the medium. Therefore, the pesticides must have accumulated at the liquid–glass interface under these conditions. Although it is possible that a slower rate of hydrolysis could occur at this interface, insulated from the effect of the hydroxyl ion, this is unlikely to explain the increased half-lives for the endosulfan isomers reported in this study with effective sealing. A similar increase in half-life was also observed for the more stable compounds such as dieldrin, when the flasks were sealed with PTFE.

This distribution to the glass–medium interface was observed when the compounds under study were added to either water or microbial growth media. An even distribution of pesticide throughout the entire system was achieved by adding 0.1% Tween 80 (Table III). All of the compounds studied were distributed throughout the system in a similar fashion, and all responded similarly to the detergent treatment in both the sterile distilled water and growth medium. There was a greater distribution of all the pesticides to the liquid–glass interface in the vessels containing pure water. An explanation for this is that the solubility of all of the solid compounds in water was much less than the levels which were applied to the vessels (1–5 $\mu g\ mL^{-1}$). These solid-phase values, from which the values in Table II are derived, are as follows: aldrin, 0.06 ppm; dieldrin, 0.18 ppm; endosulfan I, 0.33 ppm; endosulfan II, 0.41 ppm; endosulfan sulfate, 0.5 ppm; endosulfan diol, 4.8 ppm; and endosulfan ether, 14.0 ppm. The more even distribution of the cyclodiene compounds in the treatment vessels containing microbial growth medium compared with that in pure water, after the addition of Tween 80, suggests, however, that there is an additional solubilizing effect contributed by the medium. This effect would also be exerted by lipophilic constituents or dissolved organic matter in the soil, peptone, and yeast extracts. These results illustrate the importance of avoiding subsampling when aqueous extracts containing relatively high concentrations of endosulfan and related cyclodienes are analyzed.

The distribution of pesticides in aqueous systems is of particular importance in microbial degradation studies where the availability of the compound is likely to affect its degradation. Thus, when the pesticide is added in small amounts of solvent to the aqueous phase (as has generally been reported in microbial pesticide degradation studies, often followed by evaporation of solvents with N_2 gas), its distribution in the incubation vessel will tend to be associated with the interfaces. The geometry of the incubation vessel as well as the constituents of the medium will effect the pesticide distribution. In microbial degradation experiments, when the insoluble compounds are added in methanol or similar solvent, an apparent increase in pesticide concentration with time will be observed in the bulk of the medium, once exponential growth commences. This effect may be overcome by completely sacrificing the entire treatment incubation flasks at each sampling time. Maloney et al. (1988) reported on the twofold benefit of using Tween 80 in a study of the microbial degradation of the insoluble pyrethroid pesticides. It was shown to solubilize the compound to be degraded and provided the growing microorganisms with a source of readily utilizable carbon, for the transformation of the pyrethroids. Tween 80 is a mixture of oleic acid (approximately 70%) with the balance comprised of linoleic, palmitic, and stearic acids.

Significance of Water Solubility. The water solubility of the compounds studied and that of some of their metabolites have been compiled in Table II. These values, in conjunction with the octanol/water distribution coefficient, vapor-phase pressure, and pesticide polarity, are important in understanding the distribution and reactivity of these compounds in the environment (Seiber, 1987; Shui et al., 1990a,b). The relatively low solubility of all the compounds studied can account for the general distribution patterns of the pesticides observed in the incubation vessels. The slightly higher water solubilities of endosulfan II and endosulfan sulfate as compared to that of endosulfan I are a consequence of the differences in the compound's polarity. The dipole moment, which provides an indicator of a compound's polarity, has been determined by Forman et al. (1965) as 1.02, 3.18, and 4.10 Debye units for endosulfans I and II and the sulfate, respectively. The higher values for endosulfan sulfate and endosulfan II compared to that of endosulfan I correlate well with the differences in water solubility of these compounds.

The environmental significance of the low solubilities of these compounds is that they have the potential to be readily taken up by biological and other lipid-containing systems present in waters polluted with them. Accounts of the bioaccumulation of aldrin and dieldrin in a number of aquatic species are numerous, but with endosulfan, also of low water solubility, the extent of the problem is primarily with initial acute toxicity, not accumulation. The labile sulfite group necessitates a relatively rapid metabolism of the parent endosulfan isomers, to the nontoxic diol, and thereby reduces the potential of these compounds to accumulate in biological systems.

CONCLUSION

This study reports that the endosulfan isomers can be dissipated in simple aqueous systems at neutral pH in the absence of biological material or chemical catalysts. Endosulfan II is more readily degraded then endosulfan I, a phenomenon already observed in various aqueous systems. Apparent half-lives determined from the data indicate that the parent isomers are much less persistent than the related cyclodienes, aldrin and dieldrin. However, the major oxidation product of endosulfans I and II, endosulfan sulfate, is less volatile and can persist longer than either of the parent isomers in the same system. Given that endosulfan sulfate is formed in many natural environments through biological oxidation and that it is only slowly degraded, both chemically and biologically (Miles and Moy, 1978), it may represent a predominant residue of endosulfan in aerobic aqueous environments.

Endosulfan sulfate was not formed in any of the treatments. This suggests that endosulfan sulfate would not be formed in aerated waters in the absence of microbial activity or strong chemical oxidants.

The current study illustrates the importance of understanding the distribution and physical behavior of insoluble xenobiotics in aqueous systems, so that meaningful conclusions on the relative role of biological degradation in the disappearance of these compounds can be made. Even in a simple aqueous system such as that used in the current study, the apparent half-lives of the compounds studied are a function of many interacting factors. Since the compounds have very low water solubilities, their percentage distribution to the interfaces of the system will affect their rate of disappearance. The effect of the composition on the vessel's sealing material on cyclodiene disappearance was significant in all of the experiments conducted. Both endosulfan isomers disappeared from the incubation vessels at faster rates when the vessel was sealed with butyl rubber, compared with PTFE. The relatively inert PTFE seals greatly reduced losses from volatilization, thus providing the necessary conditions for studying the chemical degradation of endosulfan.

The fact that there was very little difference in the rates of dissipation of aldrin and dieldrin from media sealed with butyl rubber or unsealed showed that butyl rubber sealing was ineffective. Such a rubber seal would therefore be unsuitable for microbial degradation studies when endosulfan or other volatile cyclodienes are being examined. Although butyl rubber has a very low permeability toward O_2, it has a high affinity for organic compounds (e.g., hexane and organochlorine pesticides). On the other hand, PTFE, because of its very low coefficient of friction, has an extremely low porosity to both gases and organic volatiles (Schlanger and Baumgartner, 1980). PTFE-lined silicone rubber provides an ideal seal for anaerobic degradation studies with compounds of high volatility, and such seals have been used in anaerobic microbial degradation studies (Grbic-Galic et al., 1990).

Once there is significant microbial growth in the incubation vessel, the effect of volatilization on overall pesticide disappearance of these volatile pesticides is greatly reduced (Anderson et al., 1970; Lichtenstein et al., 1968; Wheeler, 1969). However, during the first few days of incubation or in uninoculated controls, volatilization may be significant, particularly if the system is unsealed. Therefore, one effect of the microbial growth is to stabilize the cyclodienes, reducing their fugacity in the liquid phase and thus their tendency to escape from the incubation vessel.

The concentration of 1–5 ppm of pesticide used in this study reflects the levels commonly used in studies of the microbial degradation of pesticides and in aquatic toxicology, where bioassays are performed. The relatively high proportion of pesticides distributed to the glass–media interfaces is a consequence of the small volume of the incubation vessels, as well as the low solubility of the compounds studied. When incubations are made with larger vessels, less interaction would be expected.

The results of this study are applicable to the design of studies on the microbial degradation of volatile compounds. For example, in anaerobic incubations, it is necessary to have seals which do not allow volatilization of pesticides from the growth medium, which are pliable enough to receive several punctures, and which, at the same time, prevent oxygen from entering. PTFE-lined rubber provides such a seal. Uninoculated controls could then indicate the loss from chemical degradation and not artifacts of volatilization or adsorption. In studies of aerobic biodegradation, volatilization of pesticides presents particular difficulties, because of the need to introduce air or oxygen for the respiratory needs of the cultures. If it is desired to prevent volatilization by using Teflon seals, monitoring of the oxygen concentration would be necessary, with internal absorption of carbon dioxide in alkali. In such a system a low-oxygen indicator could be included in the medium, to show if the system is becoming anaerobic, or oxygen concentration could be monitored with a suitable probe.

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