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COTTON PESTICIDES PROGRAM

FINAL REPORT

USY8 - FATE OF ENDOSULFAN IN WATER

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Ivan R. Kennedy
F. Sanchez-Bayo
J. Al-Rifai
H. Shivaramaiah
L. Hugo
A. Crossan
S. Baskaran

CRC For Sustainable Cotton Production
University of Sydney, NSW 2006

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ABSTRACT

This project tested a hypothesis that the presence of endosulfan sulphate in river water could be used as a convenient indication of river contamination by surface runoff or on dust rather than from drift or volatilisation. The following findings falsify this hypothesis:

- A mixture of α- and β- isomers of endosulfan at low concentrations (5 to 50 $\mu g\ L^{-1}$) can be converted to endosulfan sulphate in river water.
- Nevertheless, no endosulfan sulphate formation occurred in the pH range 5-9 in pure water, allowing the conclusion to be made that endosulfan sulphate formation is strictly biological; the microbial content of river water or of other biota is apparently sufficient to ensure a significant conversion rate at the low endosulfan concentrations found in river water,
- River water and laboratory media inoculated with blue-green algae degraded endosulfan at a significant rate, but no endosulfan sulphate was formed.

The project has clearly demonstrated that measurement of endosulfan sulphate concentrations in river water cannot be used as a direct indication of the extent of surface runoff. Where drift or volatilisation allows water to be contaminated to concentrations of the order of 0.1-1.0 μg/L, endosulfan sulphate formation to levels of 0.01-0.10 μg/L will be expected to occur during the next 2-3 days. At alkaline pH values of 8 or greater, the formation of equal or greater concentrations of non-toxic endosulfan diol will also occur. Formation of endosulfan sulphate rather than dissipation as endosulfan diol formation or by volatilisation from water significantly prolongs the lifetime of endosulfan residues in water because of its greater stability.

BACKGROUND REVIEW

Endosulfan is regularly found in summer as a significant contaminant in rivers in cotton growing areas of NSW by the Central and North West Regions Water Quality Program carried out by the NSW Department of Land and Water Conservation (Cooper, 1996). The chemical analyses are limited to the parent isomers and endosulfan sulphate which is a toxic breakdown product.

Endosulfan sulphate is the most frequent form of contamination of river waters with endosulfan, but when this study began, there was no clear evidence that it is formed in water. However, the degradation of endosulfan on plants and in soil has been shown to produce endosulfan sulphate (Kennedy *et al.*,1997). If there is no direct formation of endosulfan sulphate in river water, most of the endosulfan sulphate can be considered as entering the river system in run-off, either as a solution in water or carried on sediment, rather than by direct contamination by spray drift with subsequent conversion. Testing

this hypothesis could provide a critical indicator of the relative importance of the two major transport mechanisms for endosulfan of either runoff or aerial transport.

Literature data regarding endosulfan sulphate is extremely rare. Its formation by soil fungi was proposed much earlier, when isolates from soil were found capable of forming the sulphate from endosulfan. A significant paper establishing that hydrolysis of endosulfan to endosulfan diol was pH dependent (Peterson and Batley, 1993) did not examine the degradation of endosulfan sulphate. Southan and Kennedy (1995) confirmed the relationship between endosulfan hydrolysis and pH, indicating that the rate of endosulfan hydrolysis increased by a factor of ten for each increase in pH value above 7. Endosulfan sulphate breakdown was much slower, but was also pH dependent, but not as strongly related with pH as endosulfan hydrolysis.

Objectives

The main objectives of this study were:

- to determine if there are conditions under which either the α- or β- isomers of endosulfan are converted to endosulfan sulphate in water
- using unmodified samples of river water, to determine the fate of 'spiked' samples of endosulfan isomers as applied in formulations used on cotton fields and to prepare a chemical balance of the degradation pathways,
- using river water inoculated with algae/blue-green algae, to determine the fate of the endosulfan isomers.

EXPERIMENTAL

Methodology

Laboratory trials:

- Pure water, at pH 5, 7 and 9, spiked with endosulfan at 5 and 50 μg/L; water was buffered to the correct values with acetate (pH 5) and phosphate buffers (pH 7 and 9) at 5 mM concentration
- Namoi River water, sampled at Gunnedah, transported immediately to Sydney and kept cool until used (pH, turbidity determined) within 4 days of sampling; no pH buffers were added. Hawkesbury River water was also sampled in the same month and tested for endosulfan sulphate formation.
- Test for sulphate formation in river water in a laboratory incubation (light, with inoculation with algae/blue-green algae)

Laboratory protocols: All laboratory reactions and sampling were conducted as described below, adhering to the Quality Assurance protocols established in the Joint Program (Kennedy et al., 1989) and analysed by high capillary gas chromatography (Hewlett-Packard 5890A), using electron capture detection.

Reaction

- Three replicate 1L Pyrex glass culture bottles containing 0.500 L of water were spiked by adding 25 μL (5 ppb) or 250 μL (50 ppb) of 100 ppm endosulfan standard solution (2:1 proportion of α and β isomers); after spiking, the bottles were shaken vigorously by hand.
- all bottles were incubated without shaking at 30 °C
- 100 mL of dichloromethane was added to each bottle at 0, 6, 12, 24, 48 and 96 hours; at the same time 5.7 μL of 35 ppm DDD (0.2 μg) surrogate standard solution was added to each bottle; after adding solvent, bottles were shaken vigorously by hand.
- after the addition of solvent, bottles were kept in a cold room at 4 °C until extraction was completed

Extraction

- The contents of the bottles (600 mL) were separated using a 1L Teflon® funnel
- 50 mL of dichloromethane was added to wash the empty bottle and the second aliquot separated using the same funnel.
- extract was filtered through a 20 g bed of anhydrous sodium sulphate
- ca. 50 mL of hexane in 2-3 aliquots was used for washing each bottle and funnel
- each extract was concentrated using a Buchi rotary evaporator and all dichloromethane solvent replaced with hexane
- no cleanup was necessary to achieve sample analysis in all experiments

Analysis

- GC analysis was achieved using a DB17 column with a suitable temperature program
- 5.7 μL of 35.1 ppm Chlorpyrifos (0.2 μg) was used as internal standard.

Field work

Interaction was carried out with the mesocosm study of Andrew Brooks of the Department of Land and Water Conservation. This trial determined whether endosulfan sulphate was formed in field channels when spiked with endosulfan formulation in the range 0.01 – 10 μg/L. Some relevant data from this DLWC study is repeated in this report.

RESULTS & DISCUSSION

Incubations of endosulfan in pure water

The results of endosulfan incubations at buffered pH values of 9, 5 and 7 (see Fig. 1) indicate the great stability of endosulfan at pH 5, with increasing rates of disappearance at pH 7 and pH 9. This is consistent with previous data on endosulfan hydrolysis, which has been shown to be pH dependent (Peterson and Batley, 1993; Southan and Kennedy, 1995).

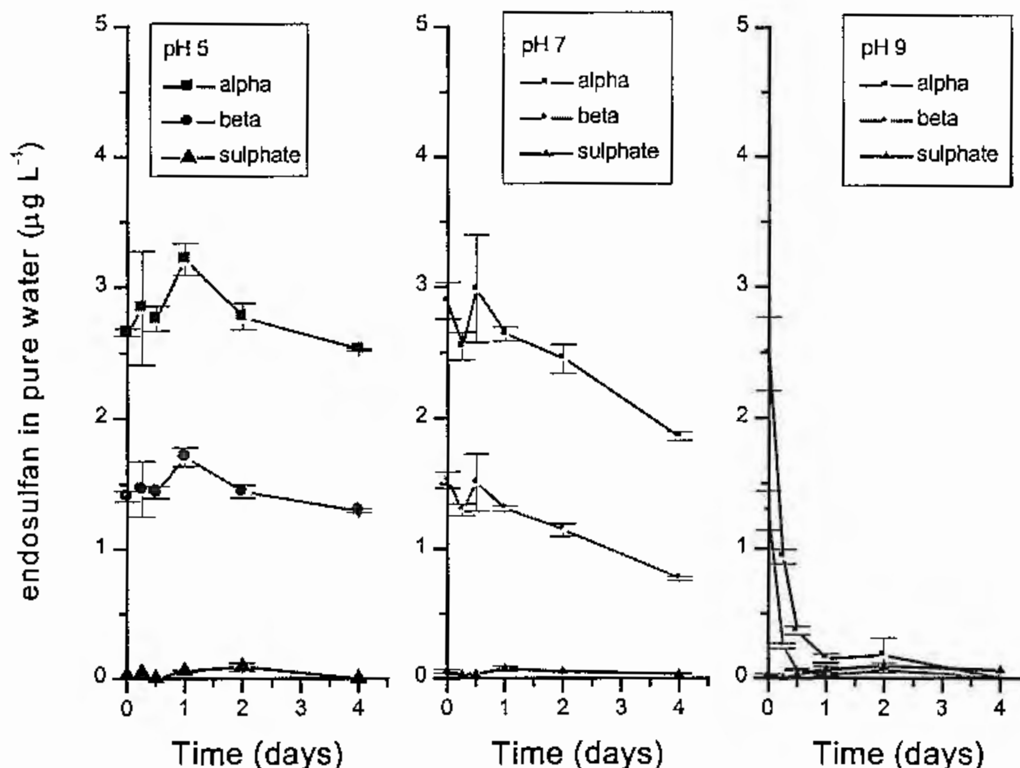


Fig. 1: Incubation of endosulfan isomers in pure water at pH 9, 5, and 7

No trend of endosulfan sulphate formation with time was observed in tests with pure water as a solvent, the values shown corresponding to the noise levels on chromatograms at the point of elution of standard endosulfan sulphate. A statistical analysis confirmed the absence of a significant trend with time and we assume that the rate of endosulfan sulphate formation in pure water, if any, is below the limit of detection in these experiments (about 0.02 ppb).

The results of these experiments are consistent with a finding that endosulfan sulphate is not formed spontaneously in pure water.

Endosulfan sulphate formation in river water

Incubations of endosulfan isomers at the pH of Namoi River water at 30°C also resulted in disappearance of endosulfan, consistent with hydrolysis to endosulfan diol as a result of more alkaline pH values but also in significant proportions of endosulfan sulphate formation (see Figure 2). A similar rate of endosulfan sulphate formation was observed using Hawkesbury River water, at 50 ppb initial endosulfan concentration (see Appendix 1). At 5 ppb, Namoi River water was more active in producing endosulfan sulphate.

With Namoi River water, the rate of endosulfan sulphate formation did not increase with higher concentration of endosulfan in water (as expected for a purely chemical reaction). On the contrary, only 2.1% conversion to endosulfan sulphate was found at the 50 ppb spiking level, while 12.6% conversion to endosulphan sulphate occurred when the initial spiked endosulfan concentration was about 5 ppb. This result is consistent with saturation of enzymatic processes at higher concentrations of endosulfan according to Michaelis-Menten kinetics, suggesting the sulphate is produced biologically.

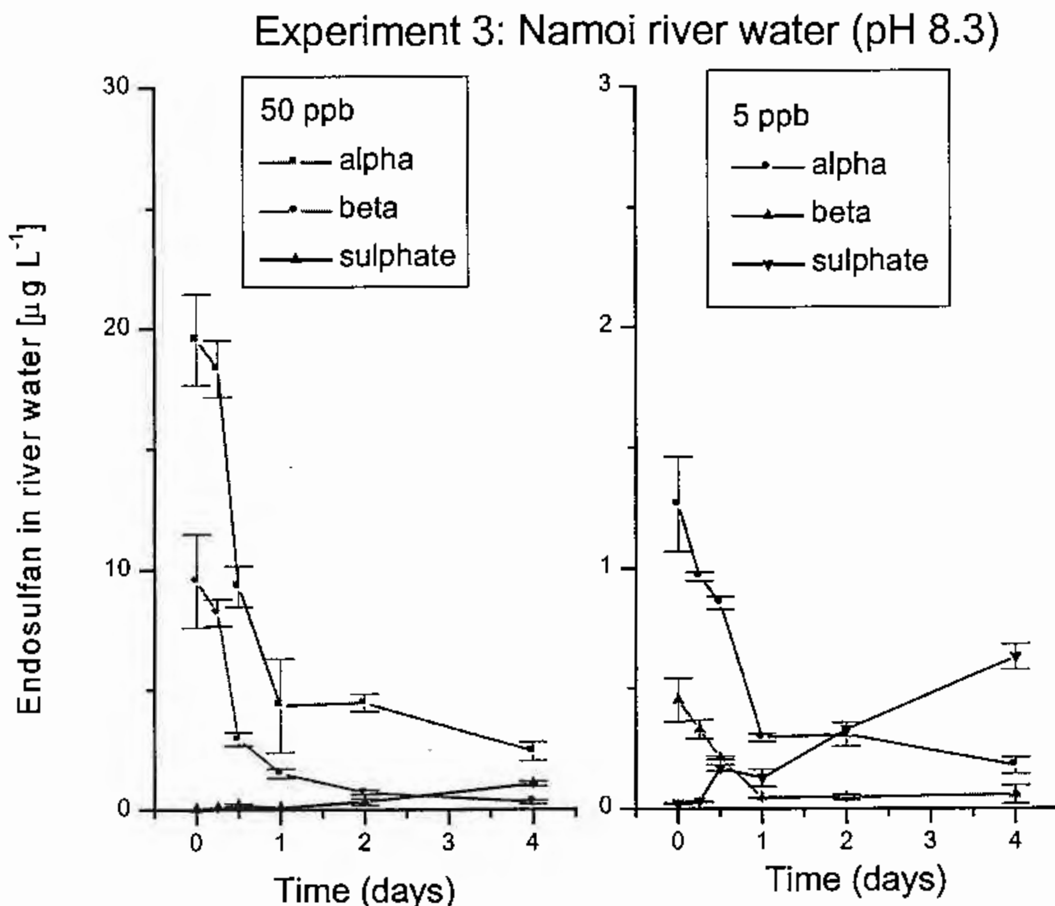


Figure 2: Dissipation of endosulfan isomers and formation of endosulfan sulphate in Namoi River water (pH 8.3)

The dissipation of both α- and β-endosulfan would be better explained by a two-phase decay curve with time, indicating that there is more than one pathway of disappearance. Possibly, some endosulfan is lost by volatilisation although precautions were taken to prevent it. There are also at least two pathways of endosulfan breakdown in non-sterile river water at pH 8.3, chemical hydrolysis to endosulfan diol and biological oxidation to endosulfan sulphate. The declining rate of endosulfan sulphate formation after the first 1-2 days is consistent with the endosulfan concentration declining to a point at which the biological reaction rate is also affected by falling endosulfan concentration.

The characteristic rate constants for endosulfan sulphate formation in Namoi River water were determined as 0.25 μg day^{-1} and 0.15 μg day^{-1} for 50 ppb and 5 ppb respectively. For Hawkesbury River water, the corresponding rates were 0.20 μg day^{-1} and 0.02 μg day^{-1} (see Appendix 1 for details).

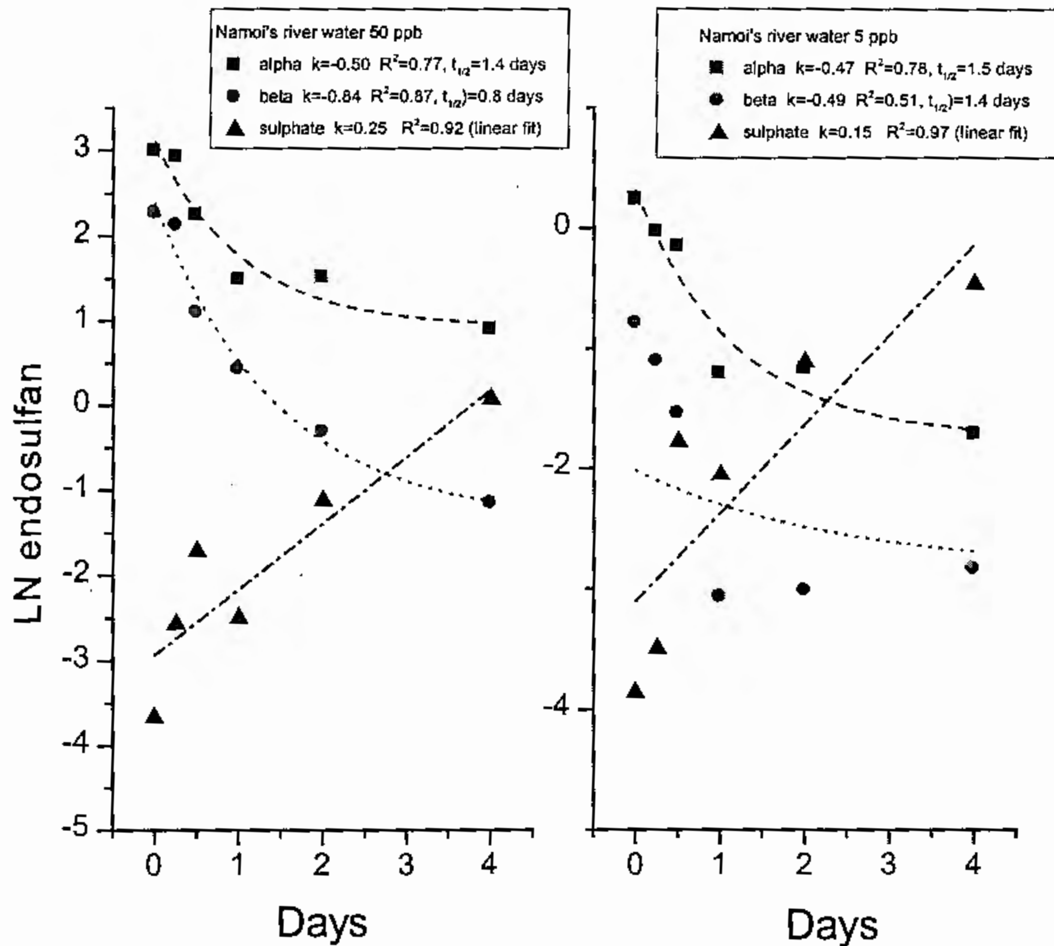


Figure 3: Logarithmic plots of endosulfan dissipation and endosulfan sulphate formation for 50 and 5 ppb solutions of endosulfan in river water

Field work

There was also clear evidence of significant endosulfan sulphate production from endosulfan formulation added to the artificial mesocosms. These mesocosms containing irrigation water obtained from the river, were shown to be inhabitated by a sizable population of micro- and meso-fauna (and presumably, microflora) at the time of endosulfan treatments. The rates of endosulfan sulphate formation with diferent spiking concentrations are shown in Figure 4.

The overall extent of conversion of endosulfan to endosulfan sulphate is similar to that observed at low concentration in the river water study above. However, a curious aspect of the data for endosulfan sulphate formation in this mesocosm study is the extremely rapid initial rate of conversion to the sulphate, with almost 50% of the maximum quantity

formed being present when the first sampling after addition was taken, just two hours later (see Fig. 4). The fact that this was directly proportional to the concentration suggests that this initial rapid rate was chemical rather than biochemical, although there is no direct evidence for this suggestion; at these concentrations, the Michaelis curve indicating extent of enzyme saturation would also be responsive to changes in endosulfan concentration. No such initial burst of activity was observed in the replicated laboratory studies with river water.

The mesocosm study also showed migration of endosulfan to the sediment of mesocosms See Fig. 5), with endosulfan being present as a significant proportion of bound sediment up to 50 hours but declining thereafter with endosulfan sulphate becoming the predominant residue species. This is consistent with the data for concentration of endosulfan residues present in the water column. This indicates that at least part of the declining rate of production of endosulfan sulphate formation in water is a result of its increased rate of migration and binding to sediments as its concentration rises with time. The other reason for this decline is the decrease in total endosulfan concentration.

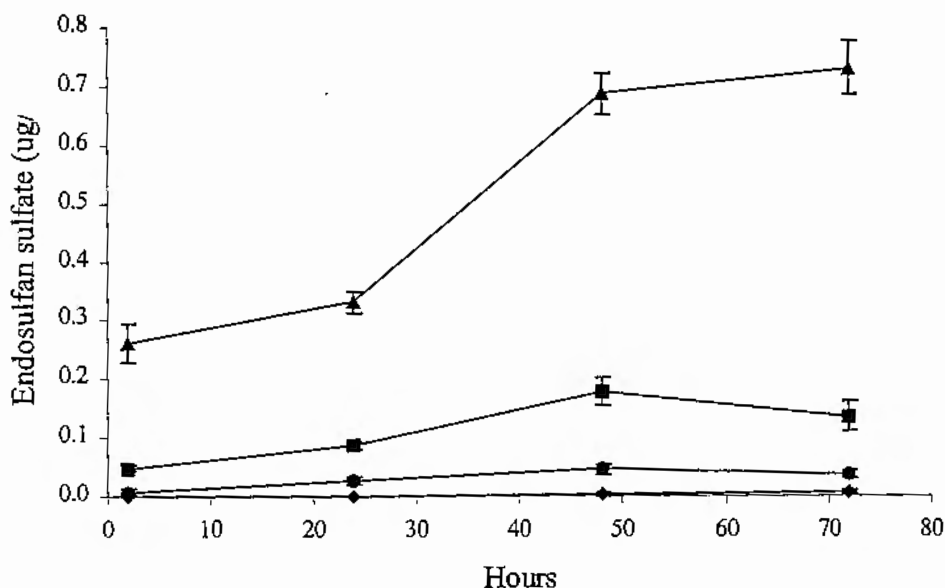


Figure 4: Formation of endosulfan sulphate in mesocosms from four levels of endosulfan application. Triangles=10µg/l nominal dose, squares =1µg/l, circles=0.1µg/l, diamonds=0.01µg/l (n=4, error bars=standard error). This is Figure 1 in the DLWC report (Brooks et al., 1998).

Whatever the source of endosulfan sulphate in these mesocosms, this study does provide clear evidence that exposed river water in the field may have a significant capacity for endosulfan sulphate formation. This was indicated in our own river water samples discussed above, although the rate of sulphate formation in river water observed was an order of magnitude less than in these field studies.

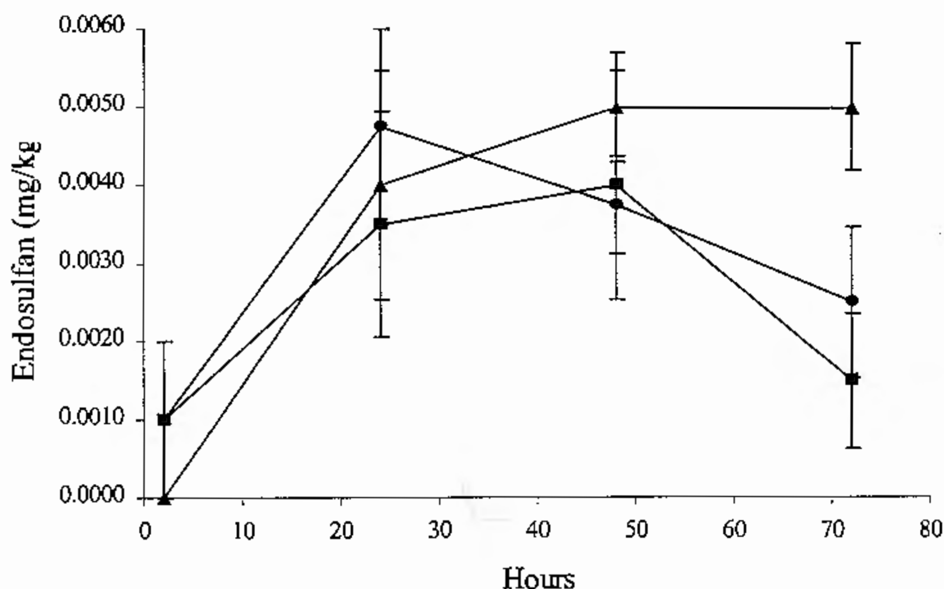


Figure 5. Mean concentrations of endosulfan in the sediment of 10μg/l mesocosms (Error bars=standard errors). α-endosulfan=circles, β-endosulfan=squares, endosulfan sulfate=triangles

Studies with cyanobacteria and a gram-negative soil bacterium

High populations of blue-green algae (cyanobacteria) failed to cause significant formation of endosulfan sulphate (Shivaramaiah and Kennedy, 1998, to be published). However, clear evidence for several other metabolites particularly endosulfan lactone (see Fig. 6), first reported by Miles and Moy (1979), was found on gas chromatograms.

The formation of endosulfan diol clearly occurs abiologically at alkaline pH values. Since cyanobacteria and other organisms may raise the pH value of growth media, some of the conversion could be chemical rather than biological. With such labile metabolites, difficult to measure accurately by gas chromatography (Guerin, Kimber and Kennedy, 1992), it is difficult to conclude what proportion of the formation of these metabolites is biological and what is purely chemical.

A gram negative bacterium isolated from cotton growing clay soils in India (Shivaramaiah, H., 1999 – in preparation) was found to catalyse the disappearance of the endosulfan isomers, but only endosulfan sulphate could be detected in the products. We can speculate that similar bacteria are present in the river water and mesocosms examined in this study. Possibly, soil bacteria capable of carrying out formation of endosulfan sulphate are ubiquitous in these soils and in river water into which soil would be eroded.

Previous failures to show formation of endosulfan sulphate in river water may reflect the low concentration necessary to obtain a significant proportion of conversion. However, these low concentrations in the range 0.01 – 1.0 μg/L are more appropriate, since they represent the levels usually encountered during the spraying season in riverine systems.

General conclusion

All the studies described in this report are consistent with the hypothesis that the formation of endosulfan sulphate is strictly biological. Indeed, the data strongly substantiate this suggestion, since there was no evidence of sulphate formation in pure water. This result is consistent with studies on the reabsorption of volatile endosulfan into shallow (5 cm deep) water trays, where little or no endosulfan sulphate formation was observed after several days (Edge and Ahmad, 1998). It is unlikely therefore, that sulphate formation is catalysed by sunlight or from some other physical or chemical factors. This conclusion was also reached in a prior study, involving ultraviolet irradiation of endosulfan isomers in films (Archer *et al.*, 1972).

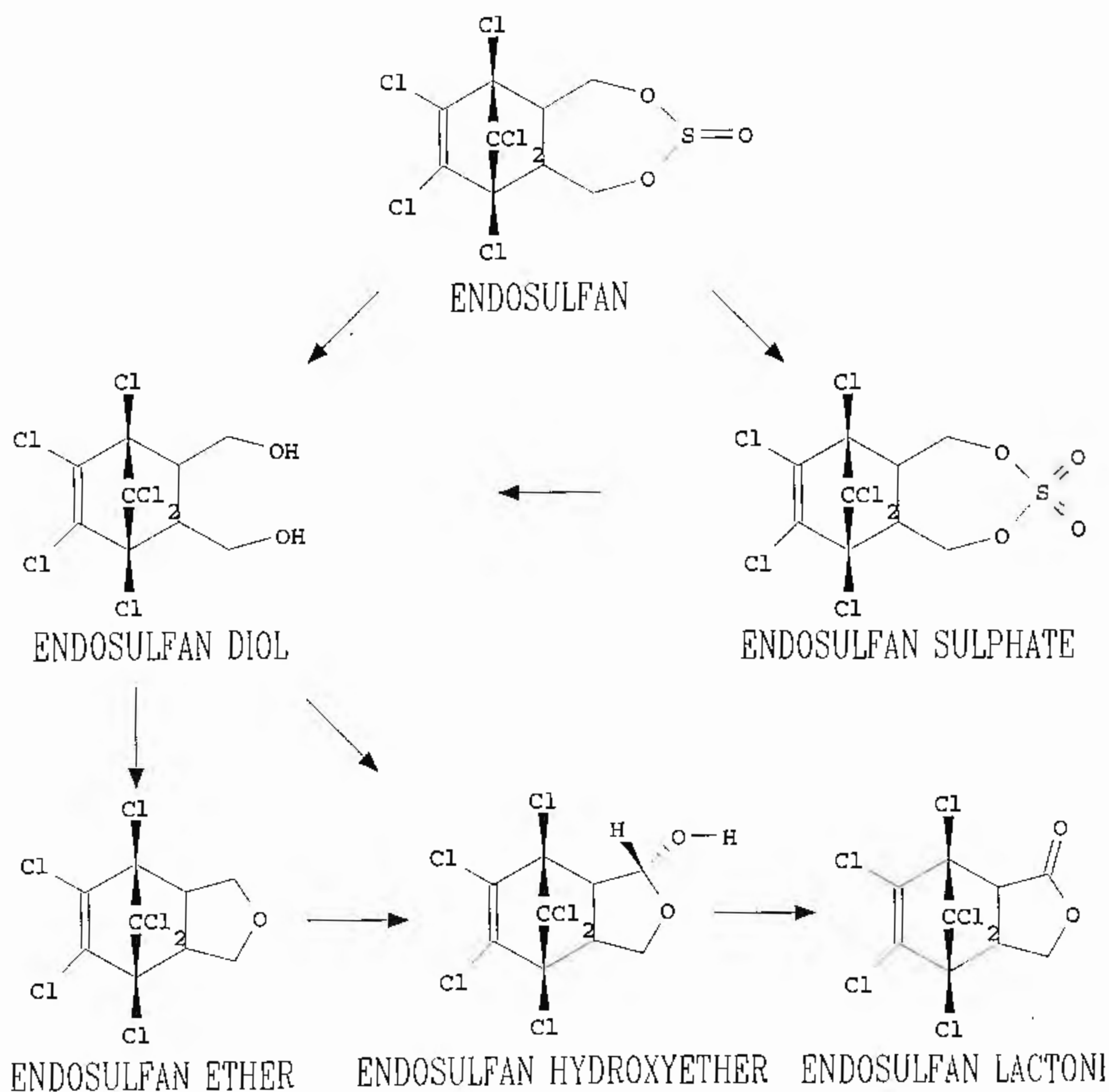


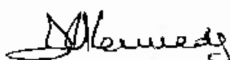
Figure 6: Metabolites of endosulfan (Miles and Moy, 1979)

There is still no clear evidence of the mechanism of endosulfan sulphate formation, although based on literature information and this study, soil bacteria or fungi seem most

likely to be concerned in a mono-oxygenase reaction. There is no evidence that blue-green algae form significant amounts of endosulfan sulphate from endosulfan, although these cyanobacteria were shown to stimulate endosulfan breakdown; whether this was caused by enzymatic catalysis, or by increased pH values that they induce in the medium, as a result of carbon and nitrogen metabolism (Kennedy, 1992), is unclear.

The minimum rate detectable of any purely chemical interconversion of endosulfan to endosulfan sulphate is determined by its limit of detection (LOD). Thus, the maximum conversion rate possible in pure water over four days at 30°C of 0.02 ppb (the experimental LOD, see Appendix 1) would have been just twice the ANZECC recommendation for endosulfan concentration in water of 0.01 μg/L. It is probable that the activation energy for formation of endosulfan sulphate is much greater than that available at this temperature.

Based on the results of this study, it can be concluded that faster biological degradation of the isomers may occur in rivers as a result of algal blooms. But endosulfan sulphate was not observed as a product of these organisms grown in laboratory culture of incubated in river water. A comprehensive literature search (STN network) and consultation with other microbial experts has failed to reveal other evidence on microbial degradation of endosulfan, or any details of the mechanism of endosulfan sulphate formation.



Ivan R. Kennedy
Professor in Agricultural & Environmental Chemistry
University of Sydney

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APPENDIX 1

Principal Investigator(s):

Ivan R. Kennedy
Professor in Agricultural & Environmental Chemistry
Department of Agricultural & Environmental Chemistry
University of Sydney, NSW 2006
Tel: 02 9351 3546 Fax: 02 9351 5108
Email: kennedy@acss.usyd.edu.au

Francisco Sanchez-Bayo
CRC Post-Doctoral Associate
Department of Agricultural & Environmental Chemistry
University of Sydney, NSW 2006

Mr. H. Shivaramaiah,
AusAID PhD Scholar,
Department of Agricultural & Environmental Chemistry
University of Sydney, NSW 2006

Data

Detection limit for sulphate ~0.02 ppb (all experiments)

1.1 Pure water experiment at low concentration (5 ppb) and pH 5, 7 and 9

Time	PH	ppb	TOTAL	alpha	beta	sulphate	TOTAL	alpha	beta	sulphate
			ppb	ppb	ppb	ppb	C/Co	C/Co	C/Co	C/Co
0 hr	5	5	4.071	2.656	1.407	0.025	1.000	0.652	0.345	0.006
6 hr	5	5	4.317	2.841	1.461	0.043	1.060	0.698	0.359	0.011
12 hr	5	5	4.197	2.761	1.436	<DL	1.031	0.678	0.353	0.000
24 hr	5	5	4.976	3.214	1.706	0.056	1.222	0.790	0.419	0.014
2 d	5	5	4.310	2.777	1.443	0.089	1.059	0.682	0.355	0.022
4 d	5	5	3.819	2.523	1.295	<DL	0.938	0.620	0.318	0.000
Time	PH	ppb								
0 hr	7	5	4.476	2.895	1.527	0.054	1.000	0.647	0.341	0.012
6 hr	7	5	3.866	2.548	1.300	0.027	0.864	0.569	0.290	0.006
12 hr	7	5	4.505	2.987	1.506	0.034	1.006	0.667	0.336	0.008
24 hr	7	5	4.024	2.642	1.309	0.073	0.899	0.590	0.292	0.016
2 d	7	5	3.646	2.449	1.144	0.053	0.815	0.547	0.256	0.012
4 d	7	5	2.651	1.858	0.770	0.035	0.592	0.415	0.172	0.008
Time	PH	ppb								
0 hr	9	5	3.803	2.490	1.291	0.034	1.000	0.655	0.339	0.009
6 hr	9	5	1.186	0.937	0.249	<DL	0.312	0.246	0.066	0.000
12 hr	9	5	0.440	0.366	0.064	0.031	0.116	0.096	0.017	0.008
24 hr	9	5	0.248	0.155	0.022	0.070	0.065	0.041	0.006	0.019
2 d	9	5	0.195	0.176	0.054	0.089	0.051	0.046	0.014	0.023
4 d	9	5	0.024	<DL	0.009	0.064	0.006	0.000	0.002	0.017

SUMMARY FOR SULPHATE

Groups	Count	Sum	Average	Variance
pH5	18	0.50	0.03	0.0016
pH7	18	0.69	0.04	0.0009
pH9	18	0.55	0.03	0.0015

Sulphate concentration was constant through time and there was no difference between pH treatments (P>0.05)

1.2 Pure water experiment at high concentration (50 ppb) and pH 5, 7 and 9

Time	PH	ppb	TOTAL	alpha	beta	sulphate	TOTAL	alpha	beta	sulphate
			ppb	ppb	ppb	ppb	C/Co	C/Co	C/Co	C/Co
0 hr	5	50	24.149	15.625	8.491	0.033	1.000	0.647	0.352	0.001
6 hr	5	50	26.700	17.274	9.368	0.087	1.106	0.715	0.388	0.004
12 hr	5	50	38.574	25.861	12.595	0.119	1.597	1.071	0.522	0.005
24 hr	5	50	30.116	19.440	10.589	0.087	1.247	0.805	0.438	0.004
2 d	5	50	39.977	26.097	13.832	0.071	1.655	1.081	0.573	0.003
4 d	5	50	36.637	24.465	12.119	0.079	1.517	1.013	0.502	0.003
Time	PH	ppb								
0 hr	7	50	29.053	19.080	9.928	0.046	1.000	0.657	0.342	0.002
6 hr	7	50	32.547	21.949	10.512	0.086	1.120	0.755	0.362	0.003
12 hr	7	50	35.079	23.567	11.437	0.076	1.207	0.811	0.394	0.003
24 hr	7	50	25.406	17.017	8.320	0.069	0.874	0.586	0.286	0.002
2 d	7	50	29.890	20.041	9.751	0.098	1.029	0.690	0.336	0.003
4 d	7	50	22.069	17.177	4.816	0.076	0.760	0.591	0.166	0.003
Time	PH	ppb								
0 hr	9	50	25.864	17.063	8.747	0.054	1.000	0.660	0.338	0.002
6 hr	9	50	9.277	6.968	2.229	0.079	0.359	0.269	0.086	0.003
12 hr	9	50	4.349	3.751	0.580	0.018	0.168	0.145	0.022	0.001
24 hr	9	50	1.561	1.445	0.095	0.022	0.060	0.056	0.004	0.001
2 d	9	50	0.435	0.394	0.020	0.022	0.017	0.015	0.001	0.001
4 d	9	50	0.066	0.009	0.024	0.034	0.003	0.000	0.001	0.001

SUMMARY FOR SULPHATE

Groups	Count	Sum	Average	Variance
pH5	18	1.43	0.079	0.0016
pH7	18	1.35	0.075	0.0014
pH9	18	0.68	0.038	0.0017

Sulphate concentration was constant thrnugh time except at pH 9 (P=0.004), at which sulphate concentration declined with time

2. River water from Namoi taken on 15 May 1998 (pH 8.3)

2.1 Experiment at low concentration (5 ppb)

2.2 Experiment at high concentration (50 ppb)

Time	ppb	TOTAL	alpha	beta	sulphate	TOTAL	alpha	beta	sulphate
		ppb	ppb	ppb	ppb	C/Co	C/Co	C/Co	C/Co
0 hr	50	29.142	19.562	9.554	0.026	1.000	0.671	0.328	0.001
6 hr	50	26.671	18.351	8.243	0.077	0.915	0.630	0.283	0.003
12 hr	50	12.468	9.331	2.958	0.178	0.428	0.320	0.102	0.006
24 hr	50	5.957	4.360	1.514	0.082	0.204	0.150	0.052	0.003
2 d	50	5.523	4.471	0.725	0.327	0.190	0.153	0.025	0.011
4 d	50	3.811	2.426	0.312	1.073	0.131	0.083	0.011	0.037
Time	ppb								
0 hr	5	1.738	1.265	0.452	0.021	1.000	0.728	0.260	0.012
6 hr	5	1.326	0.966	0.330	0.030	0.763	0.556	0.190	0.018
12 hr	5	1.237	0.856	0.212	0.169	0.712	0.493	0.122	0.097
24 hr	5	0.470	0.296	0.046	0.128	0.271	0.170	0.027	0.074
2 d	5	0.686	0.309	0.049	0.328	0.395	0.178	0.028	0.189
4 d	5	0.868	0.179	0.058	0.630	0.499	0.103	0.034	0.363

SUMMARY FOR SULPHATE

Groups	*Count*	*Sum*	*Average*	*Variance*	*Rate constant*	R^2
50 ppb*	18	5.29	0.294	0.1507	0.253	0.92
5 ppb	18	3.92	0.218	0.0488	0.151	0.97

* $P<0.05$

3. River water from Hawkesbury taken in May 1998 (pH 8.5)

3.1 Experiment at low concentration (5 ppb)

3.2 Experiment at high concentration (50 ppb)

Time	ppb	TOTAL	alpha	beta	sulphate	TOTAL	alpha	beta	sulphate
		ppb	ppb	ppb	ppb	C/Co	C/Co	C/Co	C/Co
0 hr	50	26.556	16.943	9.569	0.045	1.000	0.638	0.360	0.002
6 hr	50	19.898	13.006	6.805	0.086	0.749	0.490	0.256	0.003
12 hr	50	16.205	11.034	5.077	0.094	0.610	0.416	0.191	0.004
24 hr	50	18.951	12.318	6.462	0.171	0.714	0.464	0.243	0.006
2 d	50	12.968	8.688	4.109	0.171	0.488	0.327	0.155	0.006
4 d	50	15.114	9.574	4.943	0.896	0.569	0.361	0.186	0.034
Time	ppb								
0 hr	5	2.619	1.752	0.846	0.021	1.000	0.669	0.323	0.008
6 hr	5	1.851	1.248	0.566	0.037	0.707	0.477	0.216	0.014
12 hr	5	1.696	1.170	0.494	0.033	0.648	0.447	0.188	0.012
24 hr	5	1.971	1.370	0.568	0.034	0.753	0.523	0.217	0.013
2 d	5	1.462	1.018	0.398	0.047	0.558	0.389	0.152	0.018
4 d	5	1.092	0.728	0.238	0.126	0.417	0.278	0.091	0.048

SUMMARY FOR SULPHATE

Groups	*Count*	*Sum*	*Average*	*Variance*	*Rate constant*	R^2
50 ppb*	18	4.39	0.244	0.0970	0.201	0.88
5 ppb	18	0.89	0.049	0.0014	0.024	0.89

* $P<0.05$

APPENDIX 2

Milestone 1

One milestone was set for the project:

a) Test for endosulfan sulphate formation in river water in a laboratory incubation (dark).
b) Test for endosulfan sulphate formation in river water in a laboratory incubation (light, with inoculation of algae/blue-green algae)
c) Simulate river conditions by incubating river water in the open, with and without added algae, and spiked with endosulfan.
d) Liaise closely with mesocosm studies by Brookes and Hyne and report on additional insights.
e) Sample water from the Namoi, Gwydir and Auscott drains.
f) Undertake a comprehensive literature search and consultation with microbial experts.

ACHIEVEMENT CRITERIA: Submission of a final report to LWRRDC according to guidelines including:
a) Outcomes of literature search and consultation.
b) Summary of experimental results including
- rate of chemical interconversion to sulphate
- biological conversion to sulphate in river water
- biological conversion to sulphate in presence of algal blooms.

c) Additional fndings from related mesocosm studies.
d) Resolution of the question of and beta endosulfan sulphate in river water, rate of conversion, and implications of using sulphate as a tracer for farm sediment runoff to rivers.
e) Any publications as attachments.
Approval of the final report by LWRRDC.

APPENDIX 3: Budget

Item	$LWRRDC
Salaries	4,000
Operating (reagents, solvents, analyses)	4,500
Travel	1,500
Sub-total	10,000
	$University of Sydney
Salaries (x2, infrastructure o/heads)	$6,290 ($3145 salary IRK, 2 weeks)
Depreciation on equipment (20%/annum, etc.)	$4,444 ($200K stock equipment)
Subtotal	10,734
TOTAL	20,734

FINAL PAYMENT: $2,500 - due on approval of this Report