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Fate and Toxicity of Endosulfan in Namoi River Water and Bottom Sediment

Alex W. Leonard, Ross V. Hyne,* Richard P. Lim, Kellie A. Leigh, Jiawei Le, and Ronald Beckett

ABSTRACT

Endosulfan (6,7,8,9,10,10,-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide) sorption (standardized to 1% total organic carbon and dry weight) was significantly ($P < 0.05$) more concentrated on the large (>63 μm) particle fraction compared with smaller size fractions (<5 μm and 5–24 μm) of bottom sediments from the Namoi River, Australia. Following completion of the particle size fractionation (6 to 12 wk) and a sediment toxicity assessment (2 wk), the sediments showed large decreases in concentrations of α-endosulfan that coincided with an increase in endosulfan sulfate concentrations and minimal changes in β-endosulfan concentrations. In the Namoi River, similar patterns were observed in the composition of total endosulfan in monthly measurements of bottom sediments and in passive samplers placed in the water column following runoff from cotton (*Gossypium hirsutum* L.) fields. The toxicity of endosulfan sulfate in river water indicated by the nymphs of the epibenthic mayfly *Jappa kutera*, was more persistent than the α- and β-endosulfan parent isomers due to its longer half-life. This suggests that endosulfan sulfate would contribute most to previously observed changes in population densities of aquatic biota. Measured concentrations of total endosulfan in river water of up to 4 μg L^{-1} following storm runoff, exceed the range of the 96-h median lethal concentration (LC50) values in river water for both α-endosulfan (LC50 = 0.7 μg L^{-1}; 95% confidence interval [CI] = 0.5 to 1.1) and endosulfan sulfate (LC50 = 1.2 μg L^{-1}; 95% CI = 0.4 to 3.3). In contrast, the 10-d LC50 value for total endosulfan in the sediment toxicity test (LC50 = 162 μg kg^{-1}; 95% CI = 120 to 218 μg kg^{-1}) was more than threefold higher than the highest measured concentration of total endosulfan in field samples of bottom sediment (48 μg kg^{-1}). This suggests that pulse exposures of endosulfan in the water column following storm runoff may be more acutely toxic to riverine biota than in contaminated bottom sediment.

In the cotton-growing area of the Namoi River, Australia, the pesticide endosulfan, measured in the water column using in situ passive samplers, was found to be significantly ($P < 0.05$) correlated to decreases in population densities of mayfly nymphs and caddisfly larvae (Leonard et al., 1999, 2000). The laboratory-derived 48-h median lethal concentration (LC50) values for technical-grade endosulfan to two species of mayfly nymphs and one species of caddisfly larvae (Leonard et al., 1999) were less than the highest concentrations measured in river water following storm events (Muschal, 1998). Changes in taxon densities, which included epibenthic species, had weaker correlations to total endosulfan concentrations measured in the bottom sediment (Leonard et al., 1999). This may have been due to the more precise data obtained by using passive samplers, although the differences may also have been due to a reduced toxicity of sediment-bound endosulfan.

Evidence of runoff from land being the main route of entry of endosulfan into the river is provided by the correlation between rainfall in the locality of exposed sites and total endosulfan concentrations in the solvent of passive samplers positioned in the water column of the Namoi River (Leonard et al., 2000). We also showed previously that there was a significant regression between total endosulfan concentrations in the bottom sediment and those in the solvent of passive samplers positioned in the water column of the river (Leonard et al., 1999). This suggests that endosulfan is entering the riverine environment during storm events in both runoff water and runoff soil, as found in other studies (Antonious and Byers, 1997).

Endosulfan, a hydrophobic chemical, adheres to sediment particles in these runoff events (Chandler and Scott, 1991; Peterson and Batley, 1993; Antonious and Byers, 1997). The α- and β-isomers of endosulfan have half-lives of only a few days in water, but the biological metabolite endosulfan sulfate (Martens, 1976; Chopra and Mahfouz, 1977; Katayama and Matsumura, 1993) has an aqueous half-life of several weeks (Miles and Moy, 1979; Peterson and Batley, 1993). Both isomers and the sulfate metabolite of endosulfan are more persistent when sorbed to soil and sediment (Van Dyk and Van der Linde, 1976; Rao and Murty, 1980). Runoff from fields sprayed with endosulfan contains the α- and β-isomers plus endosulfan sulfate at concentrations toxic to nontarget aquatic biota. The α-isomer is more toxic than the β-isomer while the endosulfan sulfate is as toxic as the β-isomer (Barnes and Ware, 1965; Devi et al., 1981; Barry et al., 1995). These endosulfan compounds have distinctly different properties resulting in differences in their bioavailability and environmental fate.

Endosulfan residue studies in livers of wild fish indicate that the α-isomer is the most probable cause of

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Abbreviations: CI, confidence interval; LC50, median lethal concentration; LOEC, lowest observable effect concentration; NOEC, no observable effect concentration; TOC, total organic carbon.

toxicity to the aquatic biota in the cotton-growing region of New South Wales, Australia (Nowak and Ahmad, 1989; Nowak and Julli, 1991). However, variation in the relative proportion of endosulfan sulfate residues in fish is difficult to interpret due to metabolism of the parent isomers to endosulfan sulfate.

The bioavailability of endosulfan entering rivers would be influenced by sorptive processes onto and from the sediment particle surfaces and the distribution of the sediment-bound endosulfan in the water column (Peterson and Batley, 1993). The sediment organic-carbon content and the solubility of the compound (Neff, 1984; Forstner, 1987) largely influence sorption of organic contaminants by sediment. Particle size is the most important physical characteristic involved in the distribution of suspended sediment in the water column, thus its transport in fluvial systems (Umlauf and Bierl, 1987; Ongley et al., 1992; Newman et al., 1994).

The aim of this study was to investigate the fate and bioavailability of the parent isomers of technical endosulfan associated with the bottom sediment of the Namoi River and in river water. Both laboratory and field studies were used to investigate the fate of endosulfan and its sorption to different sizes of sediment particulate. The relative toxicity of sediment-associated and water-only exposures of the endosulfan isomers and their sulfate metabolite was evaluated in laboratory tests using the burrowing mayfly nymph, *Jappa kutera*, which is endemic to the region (Harker, 1950) and abundant in the Namoi River (Leonard et al., 2000).

MATERIALS AND METHODS

A sequence of experiments commenced with the investigation of the sorption of endosulfan compounds to different sizes of particulate within bottom sediments spiked in the laboratory as well as bottom sediments that were field-contaminated. The aim of this fractionation experiment was to give an indication of the potential bioavailability and mobility of endosulfan associated with Namoi River sediment. In the second laboratory experiment, an assessment of the bioavailability of both sediment and river water spiked with endosulfan was undertaken by toxicity testing using epibenthic nymphs of the mayfly, *J. kutera*. Trends in the fate of endosulfan compounds were described in both of these experiments. The fate of endosulfan in the laboratory experiments was compared with that in the Namoi River during the 1995–1996 spraying season for endosulfan on nearby cotton fields. The riverine samples were taken monthly and consisted of both bottom sediments and passive samplers (Peterson et al., 1995) positioned in the water column (Leonard et al., 1999).

Collection, Laboratory Spiking, and Sediment Analysis

In May 1996 and November 1996, bottom sediment recently deposited in a flood event was collected from the Namoi River in the cotton-growing region (GPS: 55J0744721) 15 km upstream of the town of Wee Waa, NSW, Australia. The two samples of sediment were press-sieved through a 1-mm mesh on-site and stored at 23°C until the laboratory-spiking procedure that occurred within 1 wk of these collections. The sediment was spiked in the laboratory with technical-grade endosulfan (Hoechst Schering AgrEvo Pty. Ltd. [Melbourne, Australia], −96% purity). The endosulfan was dissolved in acetone carrier and placed in a rolling glass jar until all the solvent had evaporated (Ditsworth et al., 1990). Wet sediment (2.5 kg) was then placed in each of the rolling jars for 3 h, with vigorous shaking every 30 min.

Concentrations of total endosulfan (α- and β-isomers plus endosulfan sulfate) in the two spiked sediment samples were measured before and after the spiking procedure. The spiked sediment (500 g) was extracted and analyzed within 4 d of spiking after storage at 4°C in solvent-rinsed brown glass jars to limit the loss of the volatile α-isomer. The sediment samples were extracted using the USEPA 3550B methodology (USEPA, 1996a) and analyzed by gas chromatography for endosulfan (USEPA, 1996b). The detection limits for all three endosulfan compounds in sediment were <0.5 μg kg^{-1} (wet weight).

In February 1997, at the end of the endosulfan cotton spraying season (November to January, inclusive) and 1 wk after a flood event, two field-contaminated bottom sediment samples were collected. One was sampled at the previous collection site in the Namoi River (GPS: 55J0744721) and the other at a site (GPS: 55J0730704) less than 20 km away at Pian Creek, a tributary of the Namoi River.

Particle Size Fraction of Sediment–Endosulfan

After taking an aliquot for chemical analysis, the two samples spiked in the laboratory with technical endosulfan and two field-contaminated samples were sent separately to the Water Studies Centre, Monash University, to be particle size fractionated using the gravitational SPLITT (split-flow lateral transport thin separation cell) fractionation technique (Springston et al., 1987). The sediment particle size fractions (based on Stokes' Law equivalent spherical diameter) collected were <5 μm, 5 to 24 μm, 25 to 63 μm, and >63 μm, as well as whole (unseparated) sediment. The May 1996 particle size fractions were analyzed whole, while all other analyses were centrifuged and only the pellets of bottom sediment were extracted (USEPA, 1996a) and analyzed for endosulfan (USEPA, 1996b). For each particle size fraction, endosulfan concentrations were expressed as μg kg^{-1} wet sediment and then standardized to dry weight and total organic carbon content. Total organic carbon of the sediment was determined using the wet oxidation and titrimetric method described by Gaudette et al. (1974).

Toxicity of Endosulfan to the Benthic Mayfly Nymph, *Jappa kutera*

In April 1997, bottom sediment was collected from the main channel of the Namoi River 10 km upstream of the cotton-growing region near the town of Gunnedah, NSW (GPS: 56J 0246971). The sediment was press-sieved through a 1-mm mesh on-site and stored at 23°C until spiking with endosulfan. Within 72 h of collection, excess water was poured off and the sediment was thoroughly mixed and spiked as described previously. Technical-grade endosulfan was added to the sediment to give nominal concentrations of 0 (solvent control), 22, 45, 90, 180, and 360 μg kg^{-1} (wet weight). Each bottom sediment treatment was replicated four times. Four aliquots (200 g) of each sediment treatment were weighed into each of the 24 one-liter test chambers (glass beakers) and covered with 600 mL of Namoi River water, to give a ratio of sediment to overlying water of 1:3 (w/v). Two days later the river water was siphoned off to remove any endosulfan not sorbed to the sediment. This was replaced with 600 mL of sonicated riverwater collected from the Namoi River upstream of the

cotton-growing region. Sonication of 2.8-L aliquots (Branson [Danbury, CT] 450 Sonifer, 100% power for 3 min) ensured dispersal of suspended particle aggregates that formed rapidly following collection (Leigh and Hyne, 1999).

Jappa kutera nymphs were collected 100 km upstream of the cotton-growing region at Lowry Ford (GPS: 56J0298924), near the town of Manilla, NSW. The length range of the mayfly nymphs used for sediment testing was 3 to 6 mm. This was a compromise between sensitivity and ease of recovery from the bottom sediment. The nymphs were transported and acclimated to laboratory conditions as described by Leonard et al. (1999).

The sediment toxicity test procedure (static, nonrenewal) followed the test procedures recommended by the American Society for Testing and Materials (1997, p. 1138–1220) for *Hexagenia* spp. mayfly nymphs. Nymphs were randomly added to each test chamber, one at a time, with the assistance of computer-generated random numbers, until each chamber contained six nymphs. All chambers were covered with clingwrap to reduce evaporation. Two air pumps were used to aerate the solutions at a rate of 50 to 100 bubbles per minute to maintain dissolved oxygen at >80% saturation. Air was supplied through Pasteur pipettes that were positioned 50 mm below the surface of the river water. The test beakers were randomly placed in an incubator set at 27 ± 1°C, on a 16:8 light–dark photocycle, with an illumination <800 cd sr m^{-2} at the surface of the test beakers. Surviving nymphs were collected by wet-sieving the sediment through a 1000-μm and a 500-μm mesh sieve. *Mobile* and *immobile* nymphs were defined as having locomotion or no locomotion in response to gentle prodding. Gill movement was not considered as a locomotory response. Missing individuals were counted as dead, as bodies would decay during the 10-d test period. Recovery of live *J. kutera* nymphs at the end of the 10-d test was more than 90% in the acetone-control treatment. In the first replicate of each treatment, temperature, pH, conductivity, dissolved oxygen, and turbidity in the overlying water were in the same range as Namoi River water, at the commencement and end of the test.

Concentrations of endosulfan in the bottom sediment (expressed as wet weight values in Fig. 1) at the commencement and at the end of the 10-d test were extracted (USEPA, 1996a) and analyzed for endosulfan (USEPA, 1996b). The total contamination and extraction efficiency, based on the nominal values, was 68% (95% CI = 62 to 74%) for the endosulfan sediment treatments.

Endosulfan concentrations in the overlying water column were determined at the end of the 10-d test. The water columns of all four replicates were pooled. The extraction and gas chromatography (GC)–electron capture detection (ECD) analyses were both duplicated and undertaken as described previously (Leonard et al., 1999). The minima and maxima contained in both duplicates were used as a basis for the error bars of measured concentrations (Fig. 1).

Toxicity testing of α-endosulfan, β-endosulfan, and endosulfan sulfate in water-only exposures was conducted using methods described in detail previously (Leonard et al., 1999), except that the nymphs were incubated in the dark. The two

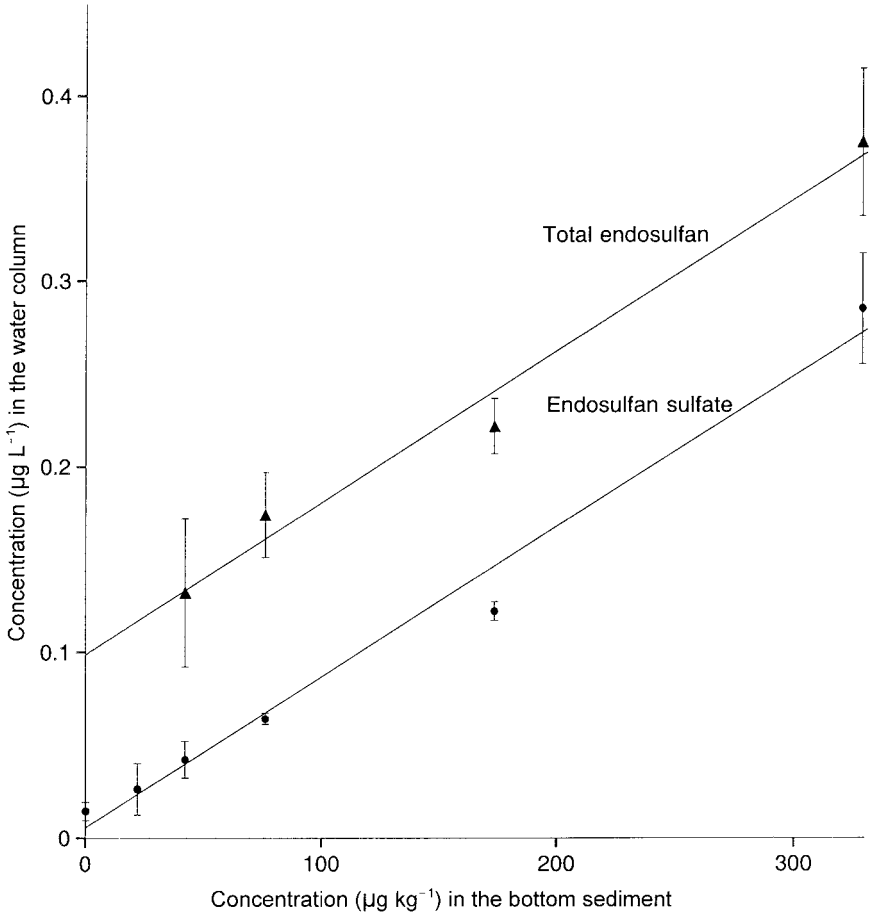

Fig. 1. Relationship between the total endosulfan (sum concentration of α- and β-endosulfan plus endosulfan sulfate) and endosulfan sulfate in the overlying water column, with total endosulfan concentrations in the bottom sediment. Both regressions for total endosulfan and endosulfan sulfate were significant ($P < 0.01$, $R^2 > 0.98$) and violated no linear assumptions.

highest treatments of endosulfan in the concentration series used in each experiment were measured after extraction using the method described above.

Pesticide Sampling in the Namoi River

Five sites along the Namoi River in the cotton-growing areas were selected because they have potentially high pesticide exposure, with the most downstream site potentially a recovery site. At each site, three soft bottom sediment samples were collected from eddies monthly between December 1995 and February 1996. The top 20 mm of sediment was transferred into brown glass jars (500 mL) that were previously rinsed with solvent and wrapped in foil. The samples were stored at 4°C for less than 4 d before they were extracted and analyzed for endosulfan (USEPA, 1996a,b). Pesticide concentrations were expressed as $\mu g\ kg^{-1}$ wet sediment. The error bars of the sediment concentrations ($\mu g\ kg^{-1}$ wet weight) in December 1995 are 95% CI values based on four sites, while the values in January and February 1996 were based on five sites (Fig. 2).

In situ passive samplers, constructed of polyethylene bags containing 10 mL of trimethylpentane, were used to quantify the bioavailable fraction of endosulfan in the water column (Peterson et al., 1995). At each sampling site, three passive samplers were placed monthly inside each of three large rock-filled nylon mesh bags (0.8 mm mesh) as described in detail previously (Leonard et al., 1999, 2000). The solvents containing the pesticides were analyzed directly by gas chromatography (GC) and confirmed using GC–mass spectrometry. The values measured at each site were pooled to give a mean value for that site. The error bars in the passive sampler measurements in December 1995 are maxima and minima values from two sites, while in January and February 1996 the error bars denote 95% confidence intervals based on four sites (Fig. 2).

Statistical Analysis

Concentrations of total endosulfan (α- and β-isomers plus endosulfan sulfate) for each particle size fraction were converted to percentage data and arc sine square root transformed. Particle size fractions that had endosulfan concentrations below the detection limit were given the maximum possible value (the detection limit). The data consisted of four replicate samples for each of the four particle size fractions. Cochran's, Hartley's, and Bartlett's test statistics and Leven's

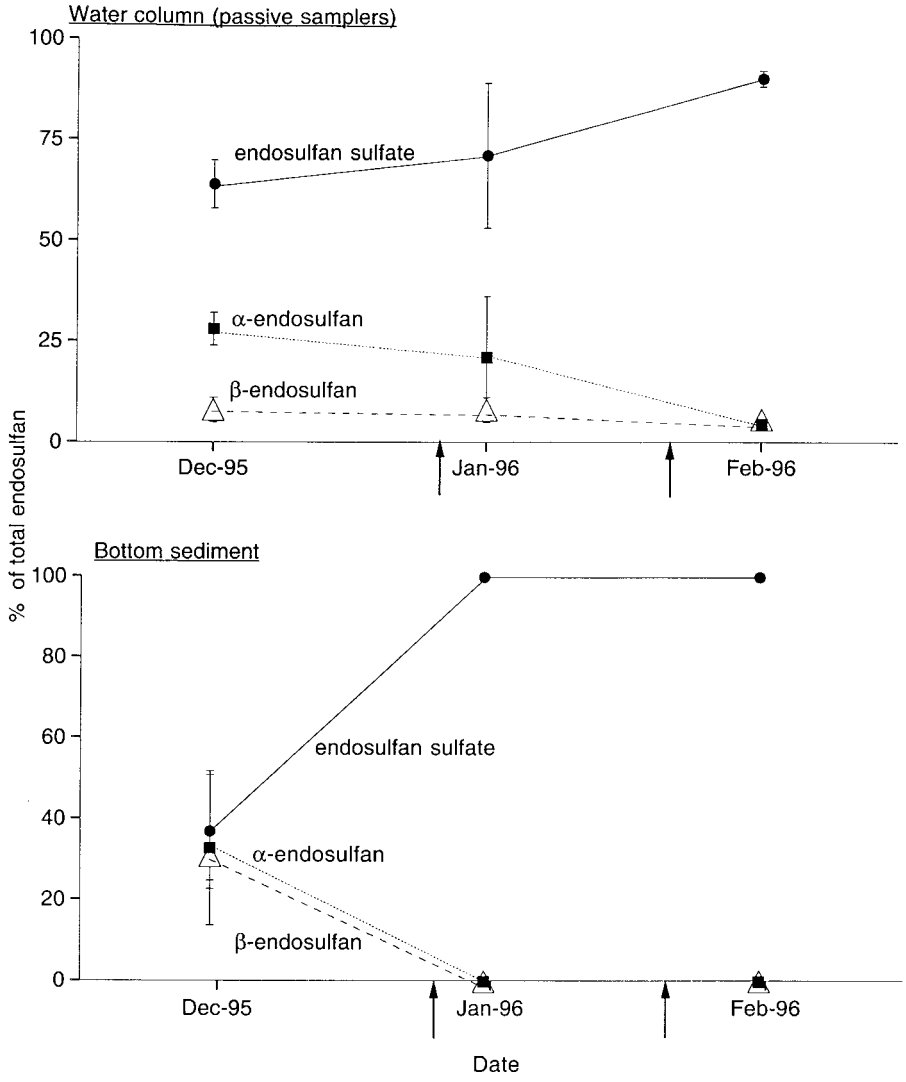

Fig. 2. Changes in the percentage composition of α-endosulfan, β-endosulfan, and endosulfan sulfate in the solvent of passive samplers placed in the water column of the Namoi River and bottom sediment samples. All sites were adjacent or downstream of cotton fields. The two vertical arrows on the *x* axis denote the approximate time of large storm events.

test indicated that variances were homogenous (Statsoft, 1997). The null hypothesis of no difference between the mean endosulfan concentration associated with each size fraction was tested using a one-way analysis of variance (ANOVA). A post-hoc Tukeys' honestly significant difference (HSD) test was used to compare probability values of similarity between each mean.

The assumptions of the regressions used in the sediment toxicity test were tested using SYSTAT software, Version 5.1 (Wilkinson, 1989). These regressions consisted of measured endosulfan concentrations in the water column against those measured in the bottom sediment. The data were square root transformed due to high residuals for the highest sediment concentrations and violated no linear assumptions. The error bars in the regressions indicate the value range based on minima and maxima of extraction duplicates and analytical duplicates (Fig. 1).

The proportional immobilization data from each water-only toxicity test were arc sine transformed and examined for normality and homogeneity of variance. If acceptable, the replicate data from each treatment were then combined and the data analyzed using Dunnett's test for comparing treatment proportions with a control proportion (Zar, 1984, p. 402–403) to obtain the lowest observable effect concentration (LOEC) and the no observable effect concentration (NOEC) values. A median lethal concentration (LC50) or a median effect concentration (EC50) value and their 95% confidence intervals (CI) were calculated, where possible, using the trimmed Spearman Karber method (Hamilton et al., 1977).

RESULTS AND DISCUSSION

Sediment Characterization

The whole bottom sediment and the particle size fractions were characterized in terms of total organic carbon (% TOC) and moisture content. The mean percentage TOC (0.7%, 95% CI = 0.5 to 1.0) was low in all samples. There were no significant differences ($P < 0.05$) in percentage TOC content between each particle size fraction and the whole sediment (data not shown). The mean moisture content of whole sediment samples had 95% CI values between 28 to 35%. The values of moisture and TOC content for sediment samples used in the sediment toxicity tests as well as the those used in the particle fractionation were not significantly different, suggesting that a similar endosulfan–sediment interaction in all experiments was likely. Unless stated otherwise, concentrations of endosulfan in sediment were standardized to 1% TOC and dry weight.

Endosulfan Decay in the Sediments during the Size Fractionation Procedure

Concentrations of endosulfan isomers and endosulfan sulfate in whole sediment were compared before and after each size fractionation procedure, which took 6 to 11 wk to complete (Table 1). For the two laboratory-spiked sediment samples treated in May and November 1996, loss of the more volatile α-endosulfan was indicated by the α/β isomer ratio being reduced from 2.6 and 2.2 immediately after spiking to <0.1 and 1.3, respectively, after completion of the fractionation procedure (Table 1). The α/β isomer ratios were <1 in the two field-contaminated samples, indicating loss of the α-isomer before collection. These ratios did not reflect the α/β isomer ratio in technical-grade endosulfan (2.3:1) that is used in the commercial spray formulation. The loss of the α-isomer may be due to volatilization and/or chemical hydrolysis of α-endosulfan in anaerobic (flooded) soils, which do not result in the formation of endosulfan sulfate (Miles and Moy, 1979). Biological oxidation of the parent endosulfan isomers leads to the formation of endosulfan sulfate, which is the major

Table 1. Change in endosulfan compounds before and after the SPLITT (Springston et al., 1987) size fractionation procedure in whole bottom sediments collected from the Namoi River catchment.

					Particle size fractionation			
					Before		After	
ESb† mode	Site	Date	FRt‡	ESx§	Dry¶	α/β#	Dry	α/β
			d		μg kg^{-1}		μg kg^{-1}	
Laboratory-spiked	Namoi River	10 May 96	47	α-ES	89	2.6	<1	<0.1
				β-ES	34		24	
				sulfate	1		10	
				ESb	125		35	
		18 Nov. 96	43	α-ES	1123	2.2	536	1.3
				β-ES	508		415	
				sulfate	61		62	
				ESb	1692		1013	
Field-contaminated	Namoi River	12 Feb. 97	79	α-ES	<1	<1.0	2	0.5
				β-ES	1		4	
				sulfate	<1		<0.7	
				ESb	3		6 to 7	
	Pian Creek	12 Feb. 97	79	α-ES	1	0.3	3	0.6
				β-ES	3		5	
				sulfate	<1		<1	
				ESb	4 to 5		7 to 8	

† ESb = total concentrations of endosulfan compounds in the bottom sediment.
‡ FRt = fractionation time.
§ ESx = endosulfan compound.
¶ Dry = dry sediments.
# α/β = ratio of α-endosulfan to β-endosulfan.

breakdown product in aerobic soils (Martens, 1976, 1977).

In the two field-contaminated samples, the measured concentrations of total endosulfan increased by approximately 3 μg kg^{-1} after the size fractionation procedure (Table 1). This suggested an error in one of the measurements and reflects the technical difficulty in measuring sediment endosulfan concentrations that are <50 μg kg^{-1} (Kennedy et al., 1998).

Endosulfan Sorption to Different Particle Size Fractions

Contact time for the sorption of endosulfan to the sediment before the particle size fractionation was between 14 and 47 d for the laboratory-spiked sediment samples and was unknown for the field-contaminated sediment samples. Testing for significant differences in endosulfan sorption between different particle size fractions was made possible by treating all four samples as replicates for each of the four particle size fractions (treatments) (Table 2). Significantly ($P < 0.01$) more endosulfan (standardized to 1% TOC and dry weight) sorbed to the largest size fraction (>63 μm) than the two smaller size fractions (<5 μm and 5 to 24 μm). The difference between the >63-μm fraction and the 25- to 62-μm size fraction was not significant ($P < 0.08$), but only marginally. These values were determined from a post-hoc Tukey's honestly significant difference (HSD) test that followed a one-way analysis of variance (ANOVA). This pattern was least evident in the laboratory-spiked sediment collected in November 1996. This may have been due to the 10-fold higher concentrations of endosulfan (1000 μg kg^{-1}) in the spiking procedure for this sample, which may have saturated the sorption capacity of the >63-μm particulate.

Endosulfan may have a greater binding affinity for a different mineral component present in the large (>63 μm) particle fraction. Or, differences in the sorptive properties of the organic carbon, not measured in its total organic carbon content, may account for the measured differences in endosulfan concentrations (Forstner, 1987; Maher et al., 1999). Sediment contaminant concentrations often decrease with increasing grain size, because specific surface areas usually decrease. However, this is not always the case, particularly if the larger particles consist of aggregates rather than primary particles. Richardson and Epstein (1971) also found higher endosulfan concentrations in the larger particle fraction (50 to 2000 μm) compared with smaller particle fractions (<0.08 μm, 0.08 μm to 0.5 μm, 0.5 to 1 μm, 1 to 2 μm, and 2 to 5 μm) in a soil spiked in the laboratory. This occurred despite the TOC content (5.0%) of the larger particle size fraction being lower than the TOC values (5.7 to 7.5%) of the smaller size fractions (Richardson and Epstein, 1971). The observation of endosulfan sorbing mainly to the larger particulate (>63 μm) of the bottom sediment has important implications for its transport and bioavailability (Umlauf and Bierl, 1987; Ongley et al., 1992; Newman et al., 1994) within the Namoi catchment. Endosulfan sorbed to large particulates of bottom sediment will only be transported under conditions of high turbulence and flow. However, desorption will occur more readily from large particles (Neff, 1984), resulting in more widespread contamination of endosulfan in river water in a dissolved (bioavailable) form.

Toxicity of Sediment-Bound Endosulfan to the Benthic Mayfly Nymph, *Jappa kutera*

The short sorption time (48 h) for laboratory spiking of sediment in the toxicity assessment of endosulfan would not have allowed equilibrium between the pore waters and the sediments (American Society for Testing and Materials, 1997, p. 1138–1220). This shorter time

Table 2. Percentage distribution of endosulfan concentrations on particle size fractions of laboratory-spiked and field-contaminated bottom sediment collected from the Namoi River catchment. Before converting to percentage data, all endosulfan concentrations were standardized to dry weight and 1% total organic carbon (TOC). < indicates minimum percent endosulfan concentration detectable by gas chromatography (GC)–electron capture detection (ECD). Endosulfan analyses of the particle size fractions were unreplicated because of the small sample sizes obtained by the size fractionation procedure.

ESb† mode	Site	Date	ESb compound	Particle size fractions (μm)			
				<5	5 to 24	25 to 63	>63
				%			
Laboratory-spiked	Namoi River	May 1996	α-ES	<4	<6	<1	7
			β-ES	<4	<6	11	31
			sulfate	<4	<6	5	17
			ESb	<11	<18	16	54
		Nov. 1996	α-ES	0	1	30	25
			β-ES	1	1	21	16
			sulfate	1	0	2	<1
			ESb	3	2	53	43
Field-contaminated	Namoi River	Feb. 1997	α-ES	<2	<2	<2	23
			β-ES	<2	<2	11	49
			sulfate	<2	<2	<2	<1
			ESb	<7	<8	12	73
	Pian Creek	Feb. 1997	α-ES	<1	<2	<1	<1
			β-ES	6	6	2	11
			sulfate	14	19	8	30
			ESb	21	26	11	41

† ESb = total concentrations of endosulfan compounds in the bottom sediment.

Table 3. Toxicity of endosulfan compounds in Namoi River water and bottom sediment to nymphs of the epibenthic mayfly, *Jappa kutera*.

				Toxicity				
							LC50††	
Phase	Length†	ESx‡	Env§	Exposure	NOEC¶	LOEC#	Mean	95% CI
	mm			μg kg^{-1}				
Sediment	5.6 to 6.0	ESb	48	10	42	76	162	120 to 218
				μg L^{-1}				
River water	4.7 to 5.0	sulfate	<4	1	0.3	0.9	3.0	2.1 to 4.2
				4	<0.3	0.3	1.2	0.4 to 3.3
		α-ES	<4	1	0.3	0.9	1.4	1.0 to 1.9
				4	<0.3	0.3	0.7	0.5 to 1.1
		β-ES	<1	1	0.9	2.4	3.9	2.8 to 5.4
				4	0.3	0.9	2.3	1.6 to 3.3
		techES‡‡		1	0.3	0.9	3.6	NR§§
				4	<0.3	0.3	1.8	NR

† The 95% confidence interval for the body lengths of nymphs used in the sediment and water tests.
‡ Endosulfan compound.
§ Highest concentration of total endosulfan measured in the region.
¶ No observable effect concentration.
# Lowest observable effect concentration.
†† Median lethal concentration.
‡‡ Technical endosulfan used for spraying (α/β = 7:3).
§§ NR indicates that the values for the 95% CI were not reliable.

used was justified, as this closely represents the fortnightly spraying frequencies in the recommended spraying strategy for cotton growing (Shaw, 1995), and intervals between the frequent storm events that occurred during the spraying season in January and February 1996 in the Namoi River valley.

The highest sediment concentration (wet weight) of total endosulfan (α- and β-isomers plus endosulfan sulfate) we measured in Namoi River sediment was 48 μg kg^{-1}, which was between the NOEC (42 μg kg^{-1}) and LOEC (76 μg kg^{-1}) values (wet weight) for *J. kutera* (Table 3). The 10-d LC50 value for total endosulfan in bottom sediment on the mayfly nymph *J. kutera* was 162 μg kg^{-1}, with a 95% CI of 120 to 218 μg kg^{-1}. These toxicity values were based on the measured total endosulfan concentrations in the bottom sediment (wet weight) at the commencement of the test. The concentrations of total endosulfan at the end of the test (Day 10) were not included because there was little change (3.4%) in the total concentration, although there were changes in the relative proportions of α- and β-endosulfan plus endosulfan sulfate.

By the end of the 10-d sediment toxicity test, the percentage composition of the α-isomer in the bottom sediments had declined by 11% (95% CI = 7 to 15%) while endosulfan sulfate had increased by 6% (95% CI = 3 to 10%), after initially being absent. In contrast, the percentage composition of the β-isomer (95% CI = 3 to 7%) increased by 5% during the 10-d sediment test. The total contamination and extraction efficiency was 68% (95% CI = 62 to 74%) for the endosulfan sediment treatments.

Concentrations of total endosulfan and endosulfan sulfate in the water column after 10 d for all treatments had significant linear regressions ($P < 0.00$, $R^2 = 0.98$) with total endosulfan in the bottom sediment (Fig. 1). This trend of increasing concentration of endosulfan sulfate in the water column with increasing concentrations of total endosulfan in the bottom sediment suggests that endosulfan sulfate was the more persistent endosulfan compound in solution, probably formed from the desorbed α-isomer. The measured concentrations of α- and β-endosulfan in the overlying water remained constant between 0.04 and 0.06 μg L^{-1} in all treatments (Fig. 1).

Endosulfan sulfate was present in the water column at the highest concentration (Fig. 1) and probably contributed significantly to the observed mortalities of *J. kutera* nymphs. Using the regression equations ($P < 0.01$; $R^2 > 0.98$) based on the data presented in Fig. 1, the highest concentration of total endosulfan in the bottom sediments (328 μg kg^{-1}) would lead to desorbed concentrations of endosulfan sulfate in the overlying water of 0.27 μg L^{-1} (95% CI = 0.25 to 0.31 μg L^{-1}) and total aqueous endosulfan of 0.37 μg L^{-1} (95% CI = 0.34 to 0.40 μg L^{-1}), after 10 d. Given the 96-h LOEC value for endosulfan sulfate (0.3 μg L^{-1}) in the river water–only exposures, the desorbed endosulfan sulfate is likely to be toxic over 10 d. The 10-d LC50, LOEC, and NOEC bottom sediment values would correspond to desorbed concentrations of total endosulfan in the overlying water of 0.23, 0.16, and 0.13 μg L^{-1}, respectively, after 10 d (Fig. 1). The intimate contact between the nymphs and the sediment would have resulted in higher exposure to the desorbed endosulfan compounds than indicated by the concentrations measured in the water column.

Relative Toxicity of Endosulfan Compounds to *Jappa kutera* Nymphs

Water-only toxicity tests in Namoi River water were undertaken to determine the relative toxicity of α-endosulfan, β-endosulfan, endosulfan sulfate, and technical-grade endosulfan, each added in single-pulse exposures to the test beakers containing *J. kutera* nymphs (Table

3). After 24 h of exposure, the α-isomer (LC50 = 1.4 μg L^{-1}) was significantly ($P < 0.05$) more toxic than the β-isomer (LC50 = 3.9 μg L^{-1}), endosulfan sulfate (LC50 = 3.0 μg L^{-1}), and the technical-grade endosulfan (LC50 = 3.6 μg L^{-1}) (Table 3). However, after 96 h the toxicity of the more persistent endosulfan sulfate (LC50 = 1.2 μg L^{-1}, 95% CI = 0.4 to 3.3) was not significantly ($P > 0.05$) different from that of the α-isomer (LC50 = 0.7 μg L^{-1}, 95% CI = 0.5 to 1.1), which in turn was significantly more toxic than the β-isomer (LC50 = 2.3 μg L^{-1}, 95% CI = 1.6 to 3.3). A 96-h LOEC value of 0.3 μg L^{-1} was obtained for α-endosulfan, endosulfan sulfate, and technical-grade endosulfan, while β-endosulfan had a LOEC of 0.9 μg L^{-1} (Table 3). No significant difference ($P > 0.05$) existed between body lengths of nymphs used in the different water-only toxicity tests. However, body lengths of these nymphs (distal region of the head to the posterior end of abdomen) (mean = 4.9 mm, 95% CI = 4.7 to 5.0) were significantly ($P < 0.05$) shorter, by approximately 1 mm, than the nymphs used in the bottom sediment toxicity test (mean = 5.8 mm, 95% CI = 5.6 to 6.0 mm) (Table 3). The sensitivity of *J. kutera* nymphs to endosulfan increases with decreasing body length (Leonard et al., 1999), but this 1-mm difference is unlikely to account for differences more than fivefold. Endosulfan in water-only toxicity tests was 150-fold more toxic to *J. kutera* nymphs than endosulfan bound to sediment.

Comparison of the toxicity data for *J. kutera* and the concentrations of endosulfan in the Namoi River for both river water and bottom sediment (Table 3) indicates that the most likely pathway for endosulfan causing adverse effects on riverine biota is in contaminated river water rather than contaminated sediment. Concentrations of total endosulfan measured in water (4 μg L^{-1}) of the nearby (<200 km) Gwydir River (Muschal, 1998) following runoff from cotton fields were significantly ($P < 0.05$) higher than the 48- to 96-h LC50 values for both α-endosulfan and endosulfan sulfate in river water (Table 3). These LC50 values in river water are consistent with our previous values for endosulfan using *J. kutera* (Leonard et al., 1999) and are similar in magnitude to other laboratory toxicity data with different species of invertebrates and fish (Sanders and Cope, 1968; Muirhead-Thomson, 1973; Sunderam et al., 1992; Hose, 2000). There is less toxicity data reported for endosulfan-contaminated sediment, although our LC50 value lies between the 4- and 12-d LC50 values reported for marine sediment toxicity assessments using a shrimp and a polychaete (McLeese and Metcalfe, 1980; McLeese et al., 1982).

In contrast to the river water, the highest concentrations of endosulfan we recorded in bottom sediment collected in the field were significantly ($P < 0.05$) lower (threefold) than the 10-d LC50 value. The concentrations of endosulfan in field sediment may have been in the range of the sediment LC50 values if smaller mayfly nymphs or endosulfan-contaminated suspended sediment was used in the toxicity test rather than bottom sediment. Concentrations of pesticides in suspended sediment have been reported to be 7-fold (Liess et al., 1996) and 15- to 43-fold (House et al., 1992) higher than that in bottom sediment.

Percentage Composition of Endosulfan Compounds in the Namoi River following Storm Events

Differences in the fate of endosulfan compounds following agricultural runoff events were measured by changes in the percentage composition of endosulfan compounds in passive samplers placed in the water column and in the bottom sediments of the Namoi River between December 1995 and February 1996 (Fig. 2). In December 1995, the percentage composition of the α-isomer in the total endosulfan measured in the water column (solvent of passive samplers) was significantly higher ($P < 0.05$) than the β-isomer (Fig. 2). In contrast, the percentages between the three endosulfan compounds measured in the bottom sediment in December 1995 were not significantly different (Fig. 2). Following two large storm runoff events in late December 1995 and late January 1996, the percentage composition of endosulfan sulfate in the total endosulfan increased significantly ($P < 0.05$) in the passive samplers collected in February 1996 and in the bottom sediment collected in January and February 1996 (Fig. 2). The increase in the percentage of endosulfan sulfate (26%) in the passive samplers coincided with a 23% decrease in the mean percentage of the α-isomer (Fig. 2). The mean percentage of β-isomer in the total endosulfan measured in the solvent of the passive samplers remained low (<8%) for all three months (Fig. 2).

In the cotton-growing regions of the Namoi, Gwydir, Border, and Macquarie Rivers, the parent isomers of endosulfan were measured in high concentrations in river water between late November and early December, but from late December to March, endosulfan sulfate dominated (Cooper, 1996). Endosulfan spraying is generally finished at the end of January (Shaw, 1995). The dominance of endosulfan sulfate in rivers after spraying may be due to its persistence in agricultural soils (Stewart and Cairns, 1974; Rao and Murty, 1980) and from wash-off of residues from the treated cotton crop (Chopra and Mahfouz, 1977; Antonious and Byers, 1997).

Both field and laboratory data on compositional changes in α- and β-endosulfan and endosulfan sulfate indicate that endosulfan sulfate probably formed from the α-isomer. Both are more likely to be mobile and toxic to aquatic biota than the β-isomer. The lower association of the α-isomer with soil or sediment particles means it is probably more bioavailable to microorganisms for metabolic oxidation to endosulfan sulfate (Martens, 1976; Katayama and Matsumura, 1993), and also more mobile during storm runoff. This was indicated by its prevalence to the β-isomer in river water (Cooper, 1996). The higher bioavailability of α-endosulfan in comparison with β-endosulfan is also indicated by the endosulfan isomer ratio in fish livers (Nowak and Ahmad, 1989; Nowak and Julli, 1991) being four-

fold higher than the isomer ratio of the technical spray mixture.

CONCLUSIONS

Endosulfan sorption was significantly greater in the largest (>63 μm) particle size fraction than in the smaller size fractions (<5 μm and 5–24 μm) for two field-contaminated sediment samples and two laboratory-spiked sediment samples. In a static laboratory sediment test, the β-isomer remained strongly sorbed to the sediment while the α-isomer desorbed, resulting in the formation of endosulfan sulfate in the water column. Endosulfan sulfate was the dominant compound measured in the total endosulfan of bottom sediment and in situ passive samplers collected from the Namoi River after land runoff events. The relatively high aqueous concentrations and persistence of endosulfan sulfate in the water column compared with the endosulfan isomers indicate that it would contribute most to any observed toxicity to nontarget aquatic biota in natural water bodies.

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